Monograph on **Gum Tragacanth** 7/31/72

M O N O G R A P H

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G U M T R A G A C A N T H

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GUM TRAGACANTH

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Summary

No information was available on the metabolism of gum tragacanth.

Riccardi and Fahrenback found that gum tragacanth fed at a level of 3% along with 3% cholesterol in the diet of cockerels inhibited the development of hypercholesterolemia (16).

Fujimoto observed that both the urethan and phenobarbital shortening of the sleeping time produced by hexobarbital administration in mice is blocked by administration of gum tragacanth (9).

Vohra and Kratzer reported that, at a 2% level in the diet, gum tragacanth results in a definite depression in the growth rate of Arbor-Acres chicks (18). Furthermore, this depressant effect is not the result of the decreased nutrient value of gum tragacanth; rather, it appears to result from some inhibitory action of the gum tragacanth on nutrient utilization or on some deleterious substance included with the gum (18).

Galbraith, et al. (10), have investigated the anti-tumorogenic activity exhibited by gum tragacanth and have found that it results from a mitotic-blocking action effected on the cellular membrane of dividing cells.

Several studies on the allergenic nature of various gums for humans have implicated gum tragacanth as being an allergen for certain susceptible individuals (5, 11, 12). At an oral dosage of 0.5 mg of gum tragacanth/kg/day for a period of 1 week, the gum tragacanth caused asthma in a susceptible subject (5).

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Chemical Information

I. Nomenclature

A. Common Names
1. Gum Tragacanth
2. Tragacanth

B. Chemical Names

Tragacanth is a complex polysaccharide mixture of tragacanthic acid (a galacturonan) and a neutral galactoaraban.

C. No Trade Names

D. CAS Registry Number PM 9000651

II. Empirical Formula

Gum tragacanth contains as the main constituent tragacanthic acid (60-70%) with a galactoarabannan as a minor polysaccharide component (3).

Treatment of tragacanthic acid with hydrochloric acid has revealed these components: D-xylose, L-fucose, D-galacturonic acid, galactose and a very small proportion of rhamnose. The relative amount of each residue is still unknown. The acid portion is associated with calcium, magnesium and potassium cations.

The neutral polysaccharide is a galactoarabannan in which the L-arabinose is by far the preponderant constituent.

Tragacanth also contains small amounts of cellulose, protein and starch.

III. Structural Formula

Tragacanthic acid contains interior chains which are almost purely a linear (1→4) linked galacturonan:

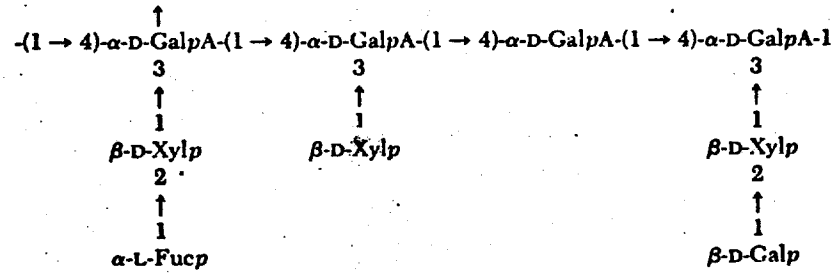
a. alpha-D-GalpA-(1→4)-D-GalpA-(1→4)-D-GalpA -- (Only the (1→4) linkages have been found although other linkages can not be ruled out). The aldobiuronic acid 2-O-(alpha-D-galactopyranosyluronic acid)-L-rhamnose (b) is a minor product of mineral acid partial hydrolysis.

b. alpha-D-GalpA-(1→2)-L-Rhap --(Only a small proportion of rhamnose residue is present). Tragacanthic acid is highly branched and evidence indicates that side chains contain only one, two or possibly, occasionally, three residues. Hydrolysis and acetolysis evidence indicates that side chains of tragacanthic acid are 2-O-alpha-L-fucopyranosyl-D-xylase (c) and 2-O-beta-D-galacto-pyranosyl-D-xylose (d). Further evidence suggests that some of these side chains may be substituted by D-glucuronic acid.

c. alpha-L-Fucp-(1→2)-D-xyl

d. beta-D-Galp-(1→2)-D-xyl

The partial structure below summarizes the main structural features of tragacanthic acid (1).



The galactoarabannan is made up of highly branched outer chains of L-arabino furanosyl residues which are involved in several types of linkage and mask the galactose core. The galactan core contains mainly (1→4) linkages, but (1→3) and (1→6) linkages may be present (2).

IV. Molecular Weight

A molecular weight of 840,000 has been determined (17).

V. Specifications

A. Chemical
See C.

B. Food
See C.

C. Food Chemicals Codex

Viscosity of a 1% solution	Not less than 250 cps
Limits of Impurities	
Arsenic	Not more than 3 ppm
Ash (total)	Not more than 3%
Ash (acid-insoluble)	Not more than .5%
Heavy metals	Not more than 40 ppm
Karaya gum	Passes test
Lead	Not more than 10 ppm

VI. Description

A. General Characteristics

Unground gum tragacanth is divided into two classifications. These are ribbons, straight or spirally twisted linear pieces, and flakes, flattened, lamellated, and frequently curved fragments. A plant will produce only ribbons or flakes and generally it is unusual for one locality to produce high-quality gums of both types. Gum tragacanth is white to weak yellow in color and translucent. It is horny in texture and it has a short fracture. It is odorless and has an insipid, mucilaginous taste. Unground gum tragacanth can be more easily pulverized when heated to a temperature of 50 degrees.

Powdered gum tragacanth is white to yellowish white in color. When it is examined microscopically in water mounts, it shows numerous fragments with circular or irregular lamellae and starch grains up to 25 microns in diameter. It shows very few or no fragments of signified vegetable tissue.

B. Physical Properties

The tragacanthic acid portion (60-70%) or bassorin is insoluble in water but swells in water to form a gel. The galactoarabannan (30-40%) or tragathan portion dissolves in water to form a colloidal hydrosol solution (14).

Viscosity is the most important factor in evaluating tragacanth. The viscosity of high grade tragacanth in a 1% solution is approximately 3400 cps. Maximum viscosity is attained after 24 hours at room temperature or by heating at 50 degrees C. for 2 hours. The viscosity of tragacanth mucilages is reduced by adding acid, alkali and NaCl, particularly if the mucilage is heated. A 4% solution has a pH of approximately 5.1-5.9; maximum viscosity is attained at pH 8.0. Maximum stability of viscosity with aging has been reported at pH 5. Gum tragacanth is relatively acid resistant, being reasonably stable down to pH 2.0.

VII. Analytical Methods

There are several methods of qualitatively identifying gum tragacanth. When a solution of the gum is boiled with a few drops of 10% aqueous ferric chloride solution, a deep yellow stringy precipitate is formed. A stringy precipitate is formed when the gum solution is heated with Schweitzer reagent (freshly precipitated copper oxide dissolved in concentrated ammonium hydroxide). A voluminous amount of translucent precipitate is formed when Millon reagent is added. Both neutral and basic lead acetate cause a precipitate which gels. A 10% solution of potassium hydroxide causes a bright yellow stringy precipitate. When gum tragacanth is placed in alcohol, it coagulates forming a long, stringy adherent. A stringy precipitate will also form when gum tragacanth is heated in concentrated sulfuric acid. Gum tragacanth gives a blue color when iodine solution is added (17).

Ewart and Chapman (6) used solubility properties to isolate the gum and developed an analytical scheme using the above precipitation and color reactions in order to qualitatively identify gum tragacanth. This scheme distinguishes between pectin, alginate, gelatin, starch, carboxymethylcellulose, methylcellulose, and several other gums, including carrageenan, tragacanth, agar, locust, karaya, ghatti and gum arabic.

Another procedure for the isolation and detection of tragacanth is to reflux the sample in 50 ml water and 50 ml of 10% by volume sulfuric acid for 3 hours. Then 30 g of barium hydroxide in 100 ml of water is added, the pH adjusted to 7, and evaporated to a small volume (4). The solution is then paper chromatographed with butanol: pyridine: water (3: 2: 1.5) as the mobile solvent and phthalic acid-aniline and naphthoresorcinol-trichloroacetic acid as developing colors. In addition to tragacanth, this method can be used to separate and identify other gums including carob bean, gum arabic, several sugars, and pectin.

Padmoyo and Miserez (15) have used microelectrophoresis to separate and identify the gums: arabic, tragacanth, carrageenan, carob and guar, as well as, gelatin, pectin starch, dextrin, agar, sodium alginate, methylcellulose and carboxymethylcellulose. The electrophoresis is carried out on cellulose acetate strips. Substances with similar motility are differentiated by staining.

VIII. Occurence

Gum tragacanth is obtained from the roots and stems of the shrub-like plants of the genus Astragalus. The principle species from which gum tragacanth is obtained is A. gummifer labillardiere. The plants thrive in dry locations in mountainous regions of Asia Minor, Iran, Syria and Turkey (17).

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Biological Data

I. Acute Toxicity

No information

II. Short-Term Studies

Chickens

One-day old Arbor-Acres chicks were weighed and separated into groups of ten. These groups were provided ad lib feed and water and weighed as a group twice weekly. At 20 or 21 days, chicks were weighed individually (18).

A control group was fed a stock diet and two test groups were fed the stock diet modified with 2% gum tragacanth. Over a period of 20 or 21 days, the two groups fed tragacanth exhibited a 34% depression of the growth rate (18).

Tragacanth at the 2% level resulted in this decreased growth rate. Furthermore, this effect was not attributable to the nutritional value of the gum itself since other non-nutritive components (cellulose) at the 2% level did not depress the growth rate (18).

III. Long-Term Studies

None

IV. Special Studies

Anti-tumorogenic

Galbraith, et al., investigated the mechanism of anti-tumorogenicity exhibited by tragacanth. Evidence was obtained that tragacanth acted by becoming attached to the cellular membrane, thereby blocking mitosis. This resulted in a degeneration of tumor cells (10).

Sensitization

A white, male patient 33 years old with a history of sneezing, rhinorrhea, and allergy to coal-tar drugs/aspirin was given pyribenzamine placebos containing gum tragacanth. He developed asthma. Scratch tests, subcutaneous injection, and neutralization tests confirmed the allergy to gum tragacanth. Very small amounts (0.5 mg/kg/day for one week) can cause severe symptoms when ingested by susceptible individuals (5).

Other studies have verified the allergenic nature of gum tragacanth for certain individuals (11, 12).

Zawahry, et al., report that tragacanth has antiallergic therapeutic value. Tragacanth powder was extracted with alcohol, and the alcoholic extract itself and the "residue" were given orally to patients suffering from dermatitis. The "residue" was markedly antiallergenic; the alcoholic extract less so (19).

An amount 150 times the therapeutic dose was given orally for 15 days without incidence of death in mice. Insufficient data were given to make an evaluation of the experimental method, results, or conclusions (19).

Fetotoxic

A 1% aqueous suspension of tragacanth when injected intraperitoneally daily between the 11th and 15th day of gestation in NMRI mice caused the death of all fetuses. This also occurred when only a single dose was administered intraperitoneally; however, this effect was not shown when injection was made subcutaneously or when administered orally (8).

Further investigation indicated that the fetotoxic effect of tragacanth was attributable to metabolic products of Enterobacter spp. contaminants in the injected tragacanth preparation (8).

Drug Interaction

Groups of male Swiss albino mice were treated intraperitoneally, subcutaneously, or orally with water, gum tragacanth, urethan, phenobarbital, and certain combinations thereof (9). Twenty-four hours after such treatment, hexobarbital sodium, 150 mg/kg, was given i.p. All drug concentrations were such that 0.1 ml of solution or suspension was given per 10 g body weight. Sleeping times to hexobarbital were measured. Numbers of mice in each group, drug, and routes of administration are indicated in the following table:

TABLE 1

Effect of Various Agents Given 24 Hours Earlier on Hexobarbital Sodium (150 mg/kg) Sleeping Time [a]

Expt.	Pretreatment, route	No. of mice	Sleeping time
A.	C	(9)	83.1±4.4
	Gt, i.p.	(10)	81.5±2.9
	GT, s.c.	(10)	90.1±4.8
	GT, p.o.	(9)	77.7±2.7
B.	C	(9)	64.7±4.6
	U, i.p.	(10)	* 45.6±1.7
	U in GT, i.p.	(10)	68.5±3.5
	U in GT, s.c.	(10)	* 51.1±4.1
	U in GT, p.o.	(10)	* 36.4±3.4
C.	C	(7)	64.3±3.5
	P, i.p.	(11)	* 38.5±2.7
	P in GT, i.p.	(9)	* 48.8±2.5
	P in GT, s.c.	(10)	* 49.8±2.4
	P in GT, p.o.	(8)	* 38.0±3.4
D.	C	(9)	74.3±6.7
	U, p.o.	(10)	* 41.7±2.5
	U, p.o. + GT, i.p.	(9)	91.3±7.3
E.	C	(10)	81.1±3.5
	P, p.o.	(10)	* 44.1±2.4
	P, p.o. + GT, i.p.	(10)	74.6±7.4
F.	C	(10)	76.7±6.2
	U, s.c.	(10)	* 48.0±5.0
	U, s.c. + GT, i.p.	(10)	81.6±5.9
G.	C	(11)	91.2±6.4
	P, s.c.	(11)	* 66.7±5.6
	P, s.c, + GT, i.p.	(11)	100.7±6.4
H.	C	(10)	68.0±6.7
	U, s.c.	(10)	* 44.3±3.6
	U, s.c. + GT, p.o.	(10)	* 46.4±3.7
	U, p.o.	(9)	* 44.4±3.8
	U, p.o. + GT, s.c.	(10)	61.7±3.3
I.	C	(11)	98.1±7.4
	P, p.o.	(10)	* 61.4±6.4
	P, p.o. + GT, s.c.	(10)	* 76.6±3.8

[a] C = control; GT = gum tragacanth, 0.1 ml/10 g body weight of a 1% suspension; U = urethan (1200 mg/kg); P = phenobarbital sodium (100 mg/kg); ± = standard error; * = P value less than 0.05 compared to control by t test.

Experiment A indicated that gum tragacanth administered i.p., s.c., or p.o. 24 hours before hexobarbital had little effect on sleeping time compared to control. Both the urethan and phenobarbital shortening of the sleeping time was blocked by administration of gum tragacanth i.p. in experiments B, D, E, F, and G. This blocking effect occurred whether the urethan and phenobarbital were given by the same route or by a different route such as the i.p. gum tragacanth. Tragacanth, having no effect on hexobarbital sleeping time, nevertheless effected a block on the effects of urethan and phenobarbital on the hexobarbital sleeping time. Gum tragacanth produced a less effective block through the s.c. route and none through the p.o. route.

Fujimoto postulated that the tragacanth block was most likely on the liver since urethan and phenobarbital shortening of hexobarbital sleeping time were hepatic effects. He further remarked that these hepatic effects were not likely to have been the result of a direct physical interaction of the tragacanth and the urethan or phenobarbital since the effect was observed in several combinations of different routes of administration (9).

On the basis of Fujimoto's data, one may also contend that the blocking effect is, in fact, a result of the physical interaction of tragacanth and urethan or phenobarbital. This contention is justified by the fact that tragacanth administered p.o. has no blocking effect; administered s.c., the tragacanth shows greater blocking activity as a result of increased incidence of a polymeric form in the blood stream. Finally, by i.p. administration, tragacanth is present in the polymeric form, thereby resulting in an active blocking effect.

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GUM TRAGACANTH

Biochemical Aspects

I. Breakdown

No information available

II. Absorption-Distribution

No information available

III. Metabolism and Excretion

No information available

IV. Effects on Enzymes and Other Biochemical Parameters

Gum tragacanth fed at a level of 3% along with 3% cholesterol in the diet of cockerels inhibited the development of hypercholesterolemia (16).

V. Drug Interaction

Both the urethan and phenobarbital shortening of the sleeping time produced by hexobarbital administration in mice is blocked by administration of gum tragacanth (9) (see biological section-special studies).

VI. Consumer Exposure Information

The major uses of gum tragacanth in the food industry include salad dressings, sauces, cheeses, ice creams, citrus oil emulsions, confections, chocolate drinks, and milk powder stabilizers (7, 14).

Gum tragacanth is listed as GRAS for use in dietary supplements and as a stabilizer.

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318. *Gum Tragacanth. Part I. Fractionation of the Gum and the Structure of Tragacanthic Acid.*

By G. O. Aspinall and J. Baillie.

Fractionation of the water-soluble part of gum tragacanth affords (i) tragacanthic acid, which contains residues of D-galacturonic acid (43%), D-xylose (40%), L-fucose (10%), and D-galactose (4%), and (ii) an arabinogalactan, which contains residues of L-arabinose (75%), D-galactose (12%), D-galacturonic acid (3%), and L-rhamnose (traces). The homogeneity of these polysaccharide preparations has been assessed by chromatography on diethylaminoethylcellulose. Controlled stepwise degradation of tragacanthic acid with acid and enzymes leads to the isolation of various oligosaccharides, including 2-*O*-α-L-fucopyranosyl-D-xylose, 2-*O*-β-D-galactopyranosyl-D-xylose, the pseudo-aldobiouronic acid, 3-*O*-β-D-xylopyranosyl-D-galacturonic acid, and oligomers of D-galacturonic acid. The main structural features of tragacanthic acid are discussed in the light of these results and of a re-examination of the cleavage products from the methylated polysaccharide.

Previous structural studies[1,2] on gum tragacanth, the exudate from various species of *Astralgus*, have shown that the gum is grossly heterogeneous and that it contains at least

[1] James and Smith, *J.*, 1945, 739.
[2] James and Smith, *J.*, 1945, 749.

two polysaccharide components, tragacanthic acid, which is composed of residues of D-galacturonic acid, D-xylose, and L-fucose,[1] and an arabinogalactan.[2] Because of difficulties in handling the gum in aqueous solution, in which only a part of it is soluble, James and Smith [1,2] carried out their structural studies on the methylated gum. They found that the methylated gum could be readily fractionated to give derivatives of tragacanthic acid, the arabinogalactan, and a glycoside of unknown structure. Some general features of the highly branched tragacanthic acid were recognised by the characterisation as methanolysis products from the methylated polysaccharide of methyl glycosides of 2,3,4-tri-*O*-methyl-L-fucose, 2,3,4-tri- and 3,4-di-*O*-methyl-D-xylose, and 2,3-di- and a mono-*O*-methyl-D-galacturonic acid; the presence of a methyl di-*O*-methyl-6-deoxy-hexoside as a further cleavage product was also suspected. In this paper we report the fractionation of the water-soluble portion of the gum and further structural studies on tragacanthic acid.

The crude gum was separated into fractions soluble and insoluble in water. Fractional precipitation of the water-soluble portion of the gum (either directly or after regeneration from the insoluble complex with Cetavlon) by addition of ethanol led to polysaccharide fractions which differed markedly in optical rotation, equivalent weight, and approximate sugar composition. Some of the fractions appeared to consist largely of either tragacanthic acid or arabinogalactan (see Table in Experimental section), but these were not sufficiently homogeneous for detailed study. The water-insoluble portion of the gum dissolved partly in dilute aqueous sodium hydroxide. The alkali-soluble material remained in solution on acidification, and graded precipitation gave fractions which were similar to those isolated from the water-soluble portion of the gum. The alkali-insoluble fraction appeared to consist largely of a glucan contaminated with adhering tragacanthic acid and arabinogalactan. It is probable that the glucan is cellulosic in nature since it remained largely insoluble after being heated with 2N-sulphuric acid but was hydrolysed by 72% sulphuric acid. The presence of cellulose microfibrils suspended in an amorphous ground substance of acidic polysaccharide has been indicated by electron microscopy for gum tragacanth and various seed mucilages.[3]

The following procedure was developed for the isolation of the two main polysaccharides in sufficiently homogeneous form for detailed studies. The crude gum was extracted with boiling ethanol to remove glycosidic components and then with cold ethanol–water (7 : 3) to afford the arabinogalactan component. The crude tragacanthic acid was most conveniently prepared as its 2-hydroxyethyl ester which was obtained by fractional precipitation of the solution resulting from treatment of the water-soluble portion of the gum with ethylene oxide.[4] Tragacanthic acid was regenerated as required from the ester by saponification with cold dilute sodium hydroxide.

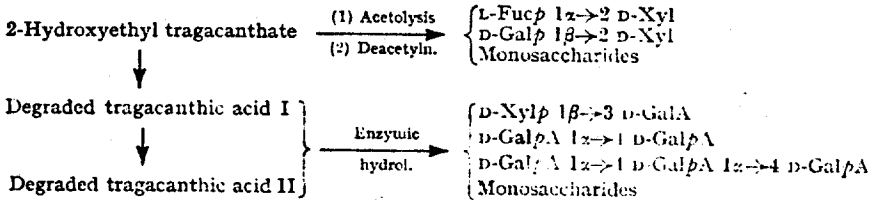
The homogeneity of the two polysaccharide preparations was assessed by glass-fibre paper ionophoresis in 2N-potassium hydroxide,[5] and by chromatography on diethylaminoethylcellulose.[6] The two polysaccharides were readily distinguished by paper ionophoresis and the individual preparations were substantially homogeneous by this criterion. Samples of each polysaccharide were then recovered by extraction of the glass paper. Paper chromatography of the hydrolysate from tragacanthic acid indicated galacturonic acid and xylose > fucose > galactose > arabinose. Subsequent experiments showed that galactose and probably also arabinose were minor constituents of tragacanthic acid and that these sugars did not arise from contaminating arabinogalactan. Likewise, chromatography of the hydrolysate from the arabinogalactan indicated arabinose > galactose > galacturonic acid > rhamnose. Rhamnose could only be detected as a constituent of the arabinogalactan. Since this sugar had not been recognised previously

[1] Mühlethaler, *Exp. Cell Res.*, 1950, **1**, 341.
[2] Deuel, *Helv. Chim. Acta*, 1947, **30**, 1523.
[3] Lewis and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 3929.
[4] Neukom, Deuel, Heri, and Kundig, *Helv. Chim. Acta*, 1960, **43**, 67.

as a constituent of gum tragacanth, a sample of the crude gum was hydrolysed and, after partial separation of the sugars formed, L-rhamnose was characterised as the toluene-*p*-sulphonylhydrazone.

When the tragacanthic acid preparation was chromatographed on diethylaminoethylcellulose the main component accounted for more than 90% of the carbohydrate material. The minor components gave arabinose, xylose, and glucose on hydrolysis and probably consisted largely of glycosidic material which had not been completely removed. The tragacanthic acid, which was recovered from the column had $[\alpha]_D$ +105° (in H_2O) and uronic anhydride content of 43%, and hydrolysis gave xylose (40%), fucose (10%), galactose (4%), and arabinose (trace). When this sample was chromatographed again on diethylaminoethylcellulose a single component was obtained.

The arabinogalactan preparation had $[\alpha]_D$ −78° (in H_2O) and uronic anhydride content of *ca.* 3%, and hydrolysis gave arabinose (75%), galactose (12%), and rhamnose (trace). When this sample was chromatographed on diethylaminoethylcellulose the main component accounted for at least 88% of the carbohydrate material; the minor components gave the same sugars in similar proportions as hydrolysis products. The arabinogalactan, which was recovered from the main peak from the column gave a single component when re-chromatographed.



Scheme showing graded hydrolysis.

2-Hydroxyethyl tragacanthate was degraded in a stepwise manner (see flow sheet). Hydrolysis of the polysaccharide ester with 0·05N-sulphuric acid on the boiling-water bath for 20 hours resulted in the release of most of the fucose residues, but only of a small proportion of the xylose and traces of the galacturonic acid residues, and degraded tragacanthic acid I was isolated. This degraded polysaccharide contained xylose and galacturonic acid residues in approximately equimolecular proportions and only traces of fucose, galactose, and arabinose residues. Under more drastic conditions (heating the polysaccharide with 0·5N-sulphuric acid on the boiling-water bath for 6 hours) further xylose residues were released and degraded tragacanthic acid II was isolated. This degraded polysaccharide had a high optical rotation ($[\alpha]_D$ +228°), approximating to those of polysaccharides of the pectic acid group.

During the early stages of the hydrolysis resulting in the formation of degraded tragacanthic acid I chromatographic examination of the products of low molecular weight indicated that neutral oligosaccharides had been released. These oligosaccharides, however, were produced more satisfactorily by acetolysis of tragacanthic acid followed by deacetylation. Two disaccharides formed in this way were isolated in pure form after chromatography on charcoal–Celite and partition chromatography on filter sheets. The first disaccharide, which was obtained crystalline, gave fucose and xylose on hydrolysis. Hydrolysis of the derived glycitol (from borohydride reduction) gave fucose and xylitol. Since the disaccharide gave no colour reaction with triphenyltetrazolium hydroxide,[1] these results pointed to the presence of a 2-*O*-fucosylxylose. The structure of the disaccharide as 2-*O*-α-L-fucopyranosyl-D-xylose was established by the isolation of 2,3,4-tri-*O*-methyl-L-fucose and 3,4-di-*O*-methyl-D-xylose on hydrolysis of the methylated disaccharide. The anomeric configuration at the glycosidic linkage may be tentatively

[1] Feingold, Avigad, and Hestrin, *Biochem. J.*, 1956, **64**, 351; Bailey, Barker, Bourne, Grant, and Stacey, *J.*, 1958, 1895.

assigned on the basis of the optical rotation of the disaccharide. In a similar manner the second disaccharide was shown to be a 2-*O*-galactosylxylose, and its structure was confirmed by the isolation of 2,3,4,6-tetra-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-D-xylose on hydrolysis of the methylated derivative. The optical rotation ($[\alpha]_D$ −40°) of the disaccharide indicated the presence of a β-D-galactopyranosyl linkage. Since this work was completed Kooiman [8] has reported the isolation of 2-*O*-β-D-galactopyranosyl-D-xylose ($[\alpha]_D$ +30°) as a partial hydrolysis product of a polysaccharide from *Tamarindus indica* seeds. This disaccharide was hydrolysed by β-galactosidase.

Two commercial enzyme preparations had little action on tragacanthic acid, and only small amounts of galactose and arabinose were liberated. In contrast, degraded tragacanthic acids I and II were extensively depolymerised with the formation of xylose, galacturonic acid, and similar series of acidic oligosaccharides. The main acidic oligosaccharide from degraded tragacanthic acid was later shown to be 3-*O*-β-D-xylopyranosyl-D-galacturonic acid, and only traces of oligomers of galacturonic acid were produced in this degradation. On the other hand, degraded tragacanthic acid II gave relatively larger proportions of di- and tri-galacturonic acids. The mixtures of acidic oligosaccharides from the two enzymic degradations were separated by paper chromatography on filter sheets, and fractions of the same chromatographic mobilities were combined. The acidic oligosaccharide with the highest mobility gave xylose and galacturonic acid on hydrolysis, whereas the derived glycitol (from borohydride reduction) furnished xylose and galactonic acid, showing the compound to be a xylosylgalacturonic acid. When the glycitol was oxidised with periodate approximately 1 mol. of formaldehyde was released, indicating that the galacturonic acid residue was 3- or 4-*O*-substituted. The acidic disaccharide was methylated, the methylated derivative was reduced with lithium aluminium hydride, and the product was hydrolysed, to give 2,3,4-tri-*O*-methyl-D-xylose and 2,4-di-*O*-methyl-D-galactose. On the basis of the optical rotation ($[\alpha]_D$ +20°) of the disaccharide a β-glycosidic linkage is indicated and the structure 3-*O*-β-D-xylopyranosyl-D-galacturonic acid may be assigned. The pseudo-aldobiouronic acid, 2-*O*-α-D-glucopyranosyl-D-glucuronic acid, has been synthesised by enzymic transglucosylation,[9] but as far as we are aware the only previously recorded isolation of a pseudo-aldobiouronic acid from the degradation of a polysaccharide is that of 4-*O*-β-D-glucosaminyl-D-glucuronic acid from hyaluronic acid.[10] The structure of the latter disaccharide has not been established directly, but has been inferred from other evidence. In contrast to aldobiouronic acids [*O*-(glycosyluronic acid)glycoses], which are very resistant to acid hydrolysis, our pseudo-aldobiouronic acid was readily cleaved by dilute acid.

The di- and tri-galacturonic acids from the enzymic hydrolysis of the degraded tragacanthic acids were isolated as calcium salts whose optical rotations ($[\alpha]_D$ +122° and +151°) were indicative of α-glycosidic linkages and were in reasonable agreement with the values quoted for the di- and tri-saccharides formed on enzymic breakdown of apple pectic acid.[11] Furthermore, the infrared spectra of the calcium salts were identical with those of the corresponding compounds isolated from lucerne pectic acid,[12] but this evidence of identity cannot be regarded as final since the spectra of the calcium salts of the di- and tri-saccharides were indistinguishable. Nevertheless, despite the absence of formal proof for the linkage between galacturonic acid residues in these compounds 1,4-linkages may be inferred since 2,3-di- and 2-*O*-methyl-D-galactose were isolated on hydrolysis of reduced methylated tragacanthic acid; the latter sugar presumably arises from the reduction of 3-*O*-β-D-xylopyranosyl-D-galacturonic acid residues.

Tragacanthic acid proved to be resistant to methylation, but samples of the polysaccharides were partly etherified by using methyl sulphate and sodium hydroxide or

[8] Kooiman, *Rec. Trav. chim.*, 1961, **80**, 849.
[9] Barker, Gomez-Sanchez, and Stacey, *J.*, 1959, 3264.
[10] Linker, Hoffman, and Meyer, *J. Biol. Chem.*, 1960, **235**, 924.
[11] Jones and Reid, *J.*, 1954, 1361.
[12] Aspinall and Fanshawe, *J.*, 1961, 4215.

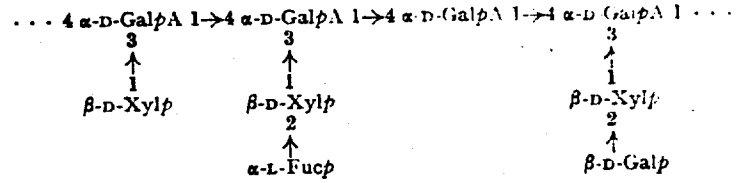
thallous hydroxide and methyl iodide, and further methylation was effected with methyl iodide and silver oxide. Methylated tragacanthic acid was reduced with lithium aluminium hydride, the reduced methylated polysaccharide was hydrolysed, and the resulting methylated sugars were fractionated by partition on cellulose. The following sugars were characterised by the formation of crystalline derivatives: 2,3,4-tri-*O*-methyl-L-fucose, 2,3,4-tri-, 3,4-di-, and 4-*O*-methyl-D-xylose, and 2,3,4,6-tetra-, 2,3,4-tri-, 2,3- and 2,4-di-, and 2-*O*-methyl-D-galactose, and some unsubstituted D-xylose and D-galactose. In addition, the following sugars, which were present only in small amounts, were provisionally characterised by one or more of the criteria, optical rotation, chromatography and paper ionophoresis of the sugars, chromatography of the products of demethylation and of periodate oxidation, and gas chromatography of the derived methyl glycosides:[13,14] di- and mono-*O*-methyl-L-fucose, di- and 3-*O*-methyl-D-xylose, 2,4,6-tri-*O*-methyl-D-galactose, and 3,5-di-*O*-methyl-L-arabinose.

With the exception of tetra- and the small proportion of 2,4,6-tri-*O*-methyl-D-galactose, the methyl ethers of D-galactose could have arisen either from D-galactose residues originally present in the polysaccharides or from D-galacturonic acid residues after reduction with lithium aluminium hydride. In view of the high proportion of D-galacturonic acid and of the low proportion of D-galactose residues in the polysaccharide, the latter alternative seemed more probable and was confirmed by the following experiments. The hydrolysis products from a sample of methylated tragacanthic acid were separated into neutral and acidic fractions. These fractions were each converted into methyl glycosides, and a portion of the acidic methyl glycosides was reduced with lithium aluminium hydride and re-treated with methanolic hydrogen chloride to ensure complete cleavage of glycosidic linkages to monosaccharide derivatives. The three mixtures of methyl glycosides from neutral sugars, acidic sugars, and reduced acidic sugars were separately examined by gas chromatography; the glycosides from the reduction of acidic sugars were also hydrolysed and the resulting sugars were examined by paper chromatography. The results showed that 2,3,4-tri-, 2,3(and probably 2,4)-di-, and 2-*O*-methyl-D-galactose were derived largely, if not exclusively, from the reduction of D-galacturonic acid residues.

The characterisation of the major neutral cleavage products from the methylated polysaccharide confirms and extends the earlier work of James and Smith[1] on tragacanthic acid. D-Xylopyranose residues occur mainly as end groups and as 2-*O*-substituted units, and L-fucopyranose residues are present largely as end groups. Since D-galactopyranose residues are present mainly as end groups it is clear that they are integral constituents of the acidic polysaccharides and that they do not arise from contaminating arabinogalactan where D-galactose residues are present in the interior chains.[2,15] Likewise, the very small proportion of L-arabinose residues in the tragacanthic acid preparation, which gives rise to 3,5-di-*O*-methyl-L-arabinose, represents a genuine minor structural feature since contamination of the polysaccharide with arabinogalactan would produce the 2,3,5-trimethyl ether as the main derivative of L-arabinose. In view of the difficulty in methylating tragacanthic acid it is possible that some of the minor cleavage products from the methylated polysaccharide are artifacts resulting from incomplete etherification. In particular, 2,4,6-tri-*O*-methyl-D-galactose, D-xylose, 4-*O*-methyl-D-xylose, and the unidentified di-*O*-methyl-L-fucose could either be products of incomplete methylation or represent genuine structural units in the interior chains of the polysaccharide. Experiments to obtain additional evidence on this and other points of fine structure are in progress.

On the basis of the results the annexed partial structure summarises the main structural features of tragacanthic acid. The polysaccharide is clearly based on essentially linear chains of 1,4-linked α-D-galacturonic acid residues. The majority of D-galacturonic acid

[13] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[14] Aspinall, *J.*, 1963, 1676.
[15] Aspinall and Baillie, following paper.



residues carry xylose-containing side-chains through C-3. Three types of side-chain have been recognised, namely, single β-D-xylopyranose residues, and disaccharide units of 2-*O*-α-L-fucopyranosyl-D-xylopyranose and 2-*O*-β-D-galactopyranosyl-D-xylopyranose, and these must account for the majority of the sugar residues in the outer chains. On the present evidence it is not possible to exclude the presence, in small proportions, of other units, *e.g.*, xylobiose units or branched side-chains. The location of the small proportion of 2-*O*-substituted L-arabinofuranose residues in the polysaccharide structure is not known.

Experimental

Paper chromatography was carried out on Whatman Nos. 1, 3MM, and 31ET papers with the following solvent systems (v/v): (A) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (B) benzene–ethanol–water (169 : 47 : 15, upper layer); (C) butan-2-one, half saturated with water; (D) ethyl acetate–pyridine–water (10 : 4 : 3); (E) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (F) ethyl acetate–acetic acid–water (10 : 5 : 6); (G) ethyl acetate–acetic acid–water (9 : 2 : 2). R_G values of methylated sugars refer to the rates of movement relative to 2,3,4,6-tetra-*O*-methyl-D-glucose in solvent A. Demethylations of methylated sugars were performed with hydriodic acid [16] or with boron trichloride.[17] Chromatography of the periodate oxidation products of methylated sugars was carried out by Lemieux and Bauer's method.[18] Unless otherwise stated, optical rotations were observed for water solutions at *ca.* 18°.

Gas–liquid partition chromatography of methylated and partially methylated methyl glycosides was carried out in a Pye argon chromatograph according to the procedure of Bishop and Cooper [13] (see also accompanying paper [14]). Separations were made on the following columns (120 × 0·5 cm.) at gas flow rates of 80—100 ml./min.: (*a*) 15% by weight of butane-1,4-diol succinate polyester [13] on acid-washed Celite (80—100 mesh) at 150°; (*b*) column *a* at 175°; (*c*) 10% by weight of polyphenyl ether [*m*-di-(*m*-phenoxyphenoxy)benzene] on acid-washed Celite at 200°. Retention times (T) are quoted relative to methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside as an internal standard.

Fractionation of Gum Tragacanth.—(i) *Fractional precipitation from aqueous solution.* Crude gum (10 g.) was dispersed in water (1 l.) for 24 hr., and the resulting suspension was diluted by the addition of water (500 ml.), stirred for 12 hr., and allowed to stand for 24 hr. at 0°. Small particles of dirt were removed by filtration through muslin, and the insoluble portion of the gum was removed by repeated centrifugation. The water-soluble portion of the gum (fraction A, 4·09 g.) was isolated by concentrating the aqueous solution and precipitating the polysaccharide by addition of ethanol containing 2% of aqueous 2N-hydrochloric acid (7 vol.). The insoluble portion of the gum (fraction B, 5·85 g.) was washed with water and dried by solvent exchange with ethanol and ether.

Fraction A (4·09 g.) was dissolved in water (500 ml.) and ethanol was added slowly with stirring. Polysaccharide fractions A1—4, which separated when the solution contained 50%, 60%, 70%, and 80% of ethanol respectively, were separated at the centrifuge. The mother-liquors after precipitation of fraction A4 were concentrated to a brown residue, which was dissolved in water and partitioned between water and butan-1-ol. The butan-1-ol layer was concentrated to a white amorphous solid, hydrolysis of which resulted in the separation of an insoluble oil and the formation of glucose, arabinose, and xylose.

Fraction B (5·85 g.) was stirred under nitrogen with N-sodium hydroxide (600 ml.) for 15 hr., and the insoluble residue (B1) was separated at the centrifuge. The alkaline solution

[16] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[17] Bonner, Bourne, and McNally, *J.*, 1960, 2929.
[18] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

was neutralised with acetic acid, and ethanol was added slowly with stirring, fractions B3 and B4 separating when the solution contained 50% and 70% of ethanol.

(ii) *Fractional precipitation after regeneration of gum fractions from Cetavlon complexes.* A saturated aqueous solution of cetyltrimethylammonium bromide ("Cetavlon") was added to a solution of fraction A (10 g.) in water (1 l.) until no further precipitate formed. The polysaccharide complex was removed at the centrifuge and washed with water. The complex was decomposed by stirring it with a 10% aqueous sodium chloride solution, and addition of ethanol gave precipitates (A5 and A6) which separated at concentrations of 40% and 70% of ethanol. The combined mother-liquor and washings from the complex were concentrated and poured into ethanol (4 vol.), to give fraction A7.

A solution of the alkali-soluble, but water-insoluble, portion of the gum (fraction B2, 10 g.) was passed through a column of Amberlite resin IR-120(H) to remove cations and treated with "Cetavlon" as above. Fraction B5 was precipitated with ethanol after regeneration of polysaccharide from the insoluble complex, and fraction B6 was precipitated with ethanol from the mother-liquors from the complex.

(iii) *Fractional precipitation of 2-hydroxyethyl esters.* Fraction A (10 g.) in water (1 l.) was deionised with Amberlite resins IR-120(H) and IR-4B(OH), and ethylene oxide (125 ml.) was added to the resulting acid solution. After 12 days the neutral solution was filtered to remove precipitate A8, which had separated, and addition of acetone gave precipitates A9 and A10 at concentrations of 20% and 50% of acetone. Fraction A9 had uronic anhydride, 41·3% (corresponding to 43·5% in the acid polysaccharide) and was used in subsequent studies on tragacanthic acid (Found: OMe, 1%; glycol ester, 5·4%).

(iv) *Extraction of the gum with ethanol–water.* Powdered gum (100 g.) was extracted with boiling ethanol for 24 hr. Concentration of the extract gave a yellow solid (3 g.) which contained unidentified glycosides and traces of glucose and arabinose. A portion of this material was chromatographed on cellulose with butan-1-ol, saturated with water, to remove monosaccharides, and hydrolysis of the resulting glycosides gave glucose, arabinose, and xylose. The ethanol-extracted gum was shaken with ethanol–water (7 : 3) for two periods of 30 hr., the mixture was centrifuged, and polysaccharide was precipitated by the addition of further ethanol. Arabinogalactan (fraction A14, 3—5 g.) was isolated after reprecipitation from aqueous solution with ethanol and had uronic anhydride, 5·4%.

(v) *Fractionation of gum fractions on diethylaminoethylcellulose.* Arabinogalactan (fraction 14, 335 mg.) was dissolved in 0·005M-sodium dihydrogen phosphate buffer (pH 6; 6 ml.) and poured on to a column (32 × 3 cm.) of diethylaminoethylcellulose (phosphate form) as described by Neukom *et al.*[6] The column was eluted with 0·025M- (500 ml.), 0·05M- (500 ml.), 0·1M- (500 ml.), and 0·25M-sodium dihydrogen phosphate (pH 6) (500 ml.), and a gradient of sodium hydroxide (0·01—0·5M; 2 l.). Fractions (*ca.* 20 ml.) were collected and analysed for sugars by the anthrone method[6] and for uronic anhydride by the carbazole method. Two minor fractions (A15 and A16) were eluted at low phosphate concentrations and were isolated after dialysis, deionisation, and concentration. The concentrated solutions were hydrolysed directly and the hydrolysates were examined by paper chromatography. The main fraction (A17, 296 mg.), which was eluted with 0·25M-phosphate, was treated in the same way and the polysaccharide was isolated by precipitation with acetone [Found: uronic anhydride, 3·2 (by decarboxylation), 5·0% (carbazole method)]. When a sample of this fraction was rechromatographed on diethylaminoethylcellulose a single peak was eluted at the same phosphate concentration.

2-Hydroxyethyl tragacanthate (fraction A9, 355 mg.) was de-esterified in 0·5M-sodium hydroxide for 3 hr., and the resulting polysaccharide was precipitated with ethanol, dissolved in 0·005M-sodium dihydrogen phosphate buffer (pH 6; 5 ml.), and chromatographed on diethylaminoethylcellulose as described previously. A number of minor fractions were eluted at low phosphate concentrations and two of these (A11 and A12) were isolated after dialysis and deionisation, and their hydrolysis products were examined by paper chromatography. The main fraction (A13, 325 mg.), which was eluted with alkali, was treated in the same way and the polysaccharide was isolated by precipitation with acetone [Found: uronic anhydride, 43·2 (by decarboxylation), 40% (carbazole method)]. When a sample of this fraction was rechromatographed on diethylaminoethylcellulose a single peak was eluted with alkali.

Examination of Fractions from Gum Tragacanth.—The results of the examination of the various fractions from gum tragacanth are given in the Table. Approximate proportions of

sugar formed on hydrolysis are indicated by +++ = strong, ++ = medium, + = weak, and tr = trace. Quantitative paper chromatography was carried out by Hirst and Jones's method.[19]

Fractions A9 (after de-esterification) and A14 were examined by ionophoresis on glass fibre paper in 2N-potassium hydroxide. The major component of A9 was immobile and only traces of other components were present. The main component was eluted from the paper and hydrolysed to give galacturonic acid, xylose, fucose, and small amounts of galactose and arabinose. The major component of A14 migrated as a discrete band and only a trace of an immobile component was present. Elution of the main component from the paper followed by hydrolysis and paper chromatography showed arabinose, galactose, and traces of galacturonic acid and rhamnose.

Examination of fractions from gum tragacanth.

Starting material	Fraction	Wt. (g.)	$[\alpha]_D$	Equiv. wt.	Sugars on hydrolysis: GalA	Gal	Ara	Xyl	Fuc	Rha	G
(i) *Fractional precipitation of the gum.*											
Crude gum (10 g.)	A1	2·81	+101°	405	+++	tr	+	+++	++	—	—
	A2	0·43	+5	—	+++	+	+	+++	+	tr	—
	A3	0·32	−35	—	+	+	++	++	—	tr	—
	A4	0·18	−50	1540	+	++	+++	+	—	tr	—
	B1 *	2·10	—	—	+	+	+	+	+	—	+
	B3	2·85	+85	575	+++	+	+	+++	+	tr	tr
	B4	0·50	−20	1100	—	++	+++	+	—	tr	—
(ii) *Fractional precipitation after regeneration of Cetavlon complex.*											
Fraction A (10 g.)	A5	6·75	+120°	405	+++	+	+	+++	++	tr	—
	A6	1·38	−40	1425	++	++	+++	+	+	tr	—
	A7	1·07	−54	1650	+	++	+++	+	—	tr	—
Fraction B2 (10 g.)	B5	7·60	+69	727	+++	+	+	+++	++	tr	tr
	B6	1·53	−21	1050	+	++	+++	+	tr	tr	—
(iii) *Fractional precipitation of 2-hydroxyethyl esters.*											
Fraction A (10 g.)	A8 *	1·78	—	—	+	tr	+	+	+	—	+
	A9	7·18	+104°	383	+++	+	tr	+++	+	tr	—
	A10	0·87	−47	1790	+	++	+++	tr	—	tr	—
(iv) *Extraction of crude gum.*											
Crude gum	A14	—	−78°	3500	3%	12%	75%	—	—	tr	—
(v) *Fractionation on diethylaminoethylcellulose.*											
Fraction A9 (0·355 g.)	A11	—	—	—	—	tr	+++	tr	—	—	++
	A12	—	—	—	tr	tr	+++	+++	—	—	+
	A13	0·325	+105°	—	43%	4%	tr	40%	10%	—	—
Fraction A14 (0·325 g.)	A15	—	—	—	tr	+++	+++	tr	—	tr	+
	A16	—	—	—	tr	+++	+++	tr	—	tr	tr
	A17	0·296	−75	—	+	+++	+++	—	—	tr	—

* Fractions B1 and A8 were incompletely hydrolysed with 2N-sulphuric acid at 100° in 18 hr. When the insoluble residue was treated with cold 72% sulphuric acid for 3 days and then with dilute sulphuric acid at 100° for 12 hr., glucose was formed as the main hydrolysis product.

Characterisation of L-*Rhamnose as a Constituent of the Gum.*—Powdered gum (10 g.) was hydrolysed with N-sulphuric acid (1 l.) at 100° for 12 hr., and the cooled suspension was filtered, neutralised with barium hydroxide, filtered again, deionised, and concentrated to a syrup (7·2 g.). The syrup was partitioned on a cellulose column (66 × 3·5 cm.) with butan-1-ol–ethanol–water (3 : 1 : 3, upper layer) as eluant. Most of the rhamnose was eluted together with fucose, xylose, and arabinose, and this fraction (540 mg.) was refractionated on cellulose (57 × 2·2 cm.) with solvent A as eluant and gave L-rhamnose (95 mg.), $[\alpha]_D$ +7° (*c* 1·9), which was characterised by conversion into the toluene-*p*-sulphonylhydrazone, m. p. 240—241° and mixed m. p. 241—242°.

Preparation of Degraded Tragacanthic Acids.—2-Hydroxyethyl tragacanthate (fraction A9, 5 g.) was heated in 0·05N-sulphuric acid (500 ml.) on the boiling-water bath for 20 hr. Ethanol

[19] Hirst and Jones, *J.*, 1949, 1659.

(5 vol.) was added to the cooled solution, and degraded tragacanthic acid I (3 g.) was removed by filtration, washed free from acid, and dried. The degraded polysaccharide had $[\alpha]_D$ +175°, equiv. 326, uronic anhydride (by decarboxylation) 46·4% (corresponding to 48·5% in the acid polysaccharide), and glycol ester 4·7%. The glycol ester content of degraded tragacanthic acids I and II was estimated by saponification and oxidation of the liberated glycol with periodate, followed by determination by the chromotropic acid reagent of the formaldehyde formed.[20] Hydrolysis of degraded tragacanthic acid I gave galacturonic acid and xylose in approximately equal proportion, and traces of fucose, galactose and arabinose. Neutralisation of the mother-liquors from the precipitation, followed by chromatography, showed fucose with smaller amounts of xylose, galactose, arabinose, and neutral oligosaccharides, and traces of galacturonic acid.

2-Hydroxyethyl tragacanthate (5 g.) was heated in 0·5N-sulphuric acid (500 ml.) on the boiling-water bath for 6 hr., and afforded degraded tragacanthic acid II (1·3 g.), $[\alpha]_D$ +228°, equiv. 202, uronic anhydride 68·5%, and glycol ester 0·5%. Hydrolysis of the degraded polysaccharide gave galacturonic acid and xylose.

Enzymic Degradation of Degraded Tragacanthic Acid.—Preliminary experiments showed that degraded tragacanthic acid I was similarly degraded by both "Hemicellulase" and "Pectinase" (L. Light and Co. Ltd.), but that the enzyme preparations had little action on tragacanthic acid and that only traces of arabinose and galactose could be detected as degradation products. Degraded tragacanthic acid I (3 g.) was treated in water (1 l.) at pH 3 (acetic acid) with "Hemicellulase" (1·2 g.) for 8 hr. Polysaccharide was precipitated with acetone (1 vol.) and was treated with enzyme for three further periods of 8 hr. The combined centrifugates were concentrated, neutralised with ammonia, and taken to dryness (2·05 g.). Paper chromatography in solvent E showed, in addition to xylose and galacturonic acid, an acidic oligosaccharide having $R_{\text{galacturonic acid}}$ 0·72 together with smaller amounts of acids having $R_{\text{galacturonic acid}}$ 0·51 and 0·38.

Degraded tragacanthic acid II (3 g.) was degraded similarly and afforded a similar mixture (1·71 g.) of monosaccharides and acidic oligosaccharides. The main oligosaccharide component had $R_{\text{galacturonic acid}}$ 0·51. The products from both enzymic hydrolyses were separated on filter sheets with solvent E, and fractions of the same mobility were combined.

Examination of Acidic Oligosaccharides.—Acidic oligosaccharide I (285 mg.), $R_{\text{galacturonic acid}}$ 0·72 in solvent E, had $[\alpha]_D$ +20° (as ammonium salt) (*c* 0·57). Hydrolysis gave xylose and galacturonic acid, and hydrolysis of the derived glycitol (from borohydride reduction) gave xylose and galactonic acid. The glycitol (10 mg.) was oxidised with 0·004M-sodium metaperiodate (25 ml.), and samples (1 ml.) were withdrawn and analysed for formaldehyde by the chromotropic acid method.[20] The formaldehyde liberated corresponded to 1 mole per mole of glycitol. The sugar (200 mg.) was methylated with methyl sulphate and 30% sodium hydroxide, care being taken to avoid strongly alkaline solutions until glycosidation was complete. The reaction mixture was acidified to pH 3 and extracted with chloroform. The methylated acid (70 mg.) was reduced with lithium aluminium hydride (150 mg.) in tetrahydrofuran (15 ml.) and furnished methylated neutral disaccharide (58 mg.). Hydrolysis of the methylated disaccharide with N-sulphuric acid for 4 hr. on the boiling-water bath gave two sugars, R_G 0·41 and 0·93, which were separated on filter sheets in solvent A. The first component (23 mg.), $[\alpha]_D$ +82° (*c* 1·1), was recrystallised from acetone containing 1% of water to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. 102° and mixed m. p. 101—102°, which was further characterised as the aniline derivative, m. p. and mixed m. p. 210°. The second component (22 mg.), $[\alpha]_D$ +20° (*c* 1·1), was recrystallised from ethanol–water, to give 2,3,4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 90—91°.

Acidic oligosaccharide II (98 mg.), $R_{\text{galacturonic acid}}$ 0·51, was chromatographically indistinguishable from the digalacturonic acid from pectic acid. It gave a calcium salt which had $[\alpha]_D$ +122° (*c* 1·2 in N-HCl) and whose infrared spectrum was identical with that of calcium digalacturonate from pectic acid.

Acidic oligosaccharide III (10 mg.), $R_{\text{galacturonic acid}}$ 0·38, gave galacturonic acid and smaller amounts of xylose on hydrolysis.

Acidic oligosaccharide IV (12 mg.), $R_{\text{galacturonic acid}}$ 0·26, was chromatographically indistinguishable from the trigalacturonic acid from pectic acid. Hydrolysis gave galacturonic acid

[20] McFadyen, *J. Biol. Chem.*, 1945, **158**, 107.

only. It gave a calcium salt which had $[\alpha]_D$ +151° (*c* 0·6 in N HCl, and an infrared spectrum identical with that of calcium trigalacturonate from pectic acid.

Acetolysis of Acetylated Tragacanthic Acid.—Acetylated tragacanthic acid (12·5 g.), prepared by the method of Carson and Maclay,[21] was added slowly to the acetolysis mixture (500 ml.; acetic acid, acetic anhydride, sulphuric acid, 10 : 10 : 1) at 0°, shaken at room temperature for 12 hr. until dissolution was complete, and set aside for a further 60 hr. The mixture was poured into water (4 l.) and sodium hydrogen carbonate was added gradually (to pH 4). The precipitated acetates were removed at the centrifuge, washed with water, and dissolved in chloroform, and the dried solution was concentrated to a syrup (8·0 g.). The syrup was dissolved in methanol (150 ml.) containing chloroform (10 ml.), 0·5N-barium methoxide (40 ml.) was added, and the mixture was shaken overnight. The resulting mixture, which was still alkaline, was poured into water (3 l.), a small insoluble residue was filtered off, the filtrate was concentrated, barium ions were removed by passage through Amberlite resin IR-120(H), and the solution was concentrated to a syrup (3·6 g.). The syrup was added to a column of charcoal (B.D.H., Ltd., acid-washed)–Celite (160 g.; 1 : 1) which was eluted successively with water, and water containing 2%, 5%, 10%, and 15% of ethanol. Each fraction was treated with Amberlite resin IR-45(OH) to remove acids, concentrated, and examined chromatographically. The fraction (454 mg.) eluted with water containing 5% of ethanol consisted of xylose and a trace of galactose. Chromatographically pure samples of the two disaccharides were obtained by fractionation on filter sheets with solvent E.

Disaccharide A (110 mg.), $R_{glucose}$ 1·4 and 1·0 in solvents D and E, was recrystallised from ethanol–water and had m. p. 185—190° (decomp.) and $[\alpha]_D$ −61° (equil.) (*c* 0·71). The sugar gave no colour with triphenyltetrazolium hydroxide and furnished fucose and xylose on hydrolysis. Hydrolysis of the derived glycitol gave fucose and xylitol. Methylation of the sugar (80 mg.) with methyl sulphate and 30% aqueous sodium hydroxide afforded methylated disaccharide (65 mg.), hydrolysis of which furnished two sugars, R_G 0·92 and 0·75, and only traces of other products. The sugars were separated on filter sheets with solvent A. The first component (28 mg.), $[\alpha]_D$ −118° (*c* 1·4), recrystallised from ethanol–water to give 2,3,4-tri-*O*-methyl-L-fucose, m. p. and mixed m. p. 62—63°, which was further characterised as the aniline derivative, m. p. 132—133°. The second component (30 mg.), $[\alpha]_D$ +21° (*c* 1·5), was chromatographically indistinguishable from 3,4-di-*O*-methyl-D-xylose and was characterised by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 67—68°.

Disaccharide B (60 mg.), $R_{glucose}$ 0·95 and 0·6 in solvents D and E, $[\alpha]_D$ +40° (*c* 3·0), gave no colour with triphenyltetrazolium hydroxide and furnished galactose and xylose on hydrolysis. Hydrolysis of the derived glycitol (from borohydride reduction) gave galactose and xylitol. Methylation of the disaccharide (55 mg.) with methyl sulphate and 30% aqueous sodium hydroxide furnished methylated disaccharide (41 mg.). Methanolysis of the methylated disaccharide afforded a mixture of methyl glycosides, the major components of which had the retention times of the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-galactose (*T* 1·86sh, 1·99) and 3,4-di-*O*-methyl-D-xylose (*T* 1·32, 1·63) when examined by gas chromatography on column *a*. The major portion of the methylated disaccharide was hydrolysed to give two sugars, R_G 0·88, 0·75, which were separated on filter sheets with solvent A. The first component (18 mg.), $[\alpha]_D$ +118° (*c* 0·91), was characterised as 2,3,4,6-tetra-*O*-methyl-D-galactose by conversion into the aniline derivative, m. p. and mixed m. p. 188—189°. The second component (17 mg.), $[\alpha]_D$ +23° (*c* 0·85), was chromatographically indistinguishable from 3,4-di-*O*-methyl-D-xylose and was characterised by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 67—68°.

Preparation and Hydrolysis of Reduced Methylated Tragacanthic Acid.—Tragacanthic acid proved very resistant to methylation, and samples of partially methylated polysaccharide (Found: OMe, *ca.* 30%) were obtained by two procedures. In the first procedure the water-soluble portion of the gum was methylated with methyl sulphate and 30% aqueous sodium hydroxide, methylated glycoside and methylated arabinogalactan were removed by extraction with chloroform, and partially methylated tragacanthic acid was isolated after dialysis and concentration of the remaining solution. This material was converted into the silver salt and treated with methyl iodide and silver oxide in methanol suspension. In the second procedure, 2-hydroxyethyl tragacanthate was converted into the thallium salt with thallous hydroxide and treated with methyl iodide in methanol suspension. The combined samples of partially

[21] Carson and Maclay, *J. Amer. Chem. Soc.*, 1946, **68**, 1015.

methylated tragacanthic acid were further methylated by several treatments with methyl iodide and silver oxide; methanol and later acetone and tetrahydrofuran were added to aid solution. Methylated tragacanthic acid had $[\alpha]_D$ +90° (*c* 0·98 in $CHCl_3$) (Found: OMe, 38·6%, not raised on further methylation).

Lithium aluminium hydride (3 g.) in tetrahydrofuran (25 ml.) was added dropwise to methylated tragacanthic acid (2·8 g.) in tetrahydrofuran (150 ml.), and the mixture was refluxed for 2 hr. Further hydride (1 g.) was added, and the mixture was refluxed for 1 hr., and set aside for 18 hr. The excess of hydride was destroyed by addition of ethyl acetate, and the solution was brought to pH 4 by addition of dilute sulphuric acid. The solution was extracted with chloroform, the extract was dried and concentrated, and reduced methylated tragacanthic acid (1·9 g.) was precipitated by light petroleum. A further quantity of methylated polysaccharide was isolated from the aqueous layer after neutralisation, concentration, and extraction of the dry residue with chloroform. Reduced methylated tragacanthic acid (2·15 g.) had $[\alpha]_D$ +78° (*c* 1·2 in $CHCl_3$) (Found: OMe, 31·8%). Hydrolysis of a sample of the methylated polysaccharide followed by paper chromatography of the hydrolysate failed to reveal methylated uronic acids.

Reduced methylated tragacanthic acid (1·9 g.) was dissolved in N-hydrochloric acid (125 ml.) and after 2 days the solution was gradually warmed to 100°, and the hydrolysis was completed by heating the mixture on the boiling-water bath for 10 hr. (constant rotation). The solution was neutralised with silver carbonate and concentrated to a syrup (1·7 g.) which was separated on cellulose (74 × 4 cm.), (i) light petroleum (b. p. 100—120°)-butan-1-ol (4 : 1, later 7 : 3, and 1 : 1), saturated with water, (ii) butan-1-ol, half saturated with water, and (iii) water being

Analysis of hydrolysate of reduced methylated tragacanthic acid.

Fraction	Wt. (mg.)	$[\alpha]_D$	Paper chromatography * R_G	Sugar	Sugars given on demethyln.	Other evidence †
1	397	+16°	0·93	2,3,4-Me_3xylose	Xylose	B, C, D
			0·88	2,3,4-Me_3fucose (*t*)		
2	131	−80	0·93	2,3,4-Me_3xylose	Xylose	B, C, D
			0·88	2,3,4-Me_3fucose	Fucose	
3	148	−12	0·88	Me_4galactose	Galactose	B, C, D
			0·81	Me_2fucose	Fucose	
			0·75	3,4-Me_2xylose	Xylose	
4	172	+14	0·93	2,3,4-Me_3xylose (*t*)		B, C, D, I
			0·87	2,3,4-Me_3fucose (*t*)	Xylose	
			0·80	Me_2fucose (*t*)	Fucose (*t*)	
			0·75	3,4-Me_2xylose		
5	32	+43	0·75	3,4-Me_2xylose (*t*)	Xylose	B, C, D, I
			0·68	Me_3galactose	Galactose	
6	19	+65	0·68	Me_3galactose	Galactose	B
			0·56	Me fucose (*t*)	Fucose (*t*)	
7	20	+16	0·68	Me_3galactose	Galactose	B, C
			0·68	Me_2xylose	Xylose	
			0·56	Me fucose (*t*)	Fucose (*t*)	
8	21	+32	0·50	Me fucose	Fucose; Galactose (*t*)	B, C, I, P
9	22	+66	0·55	Me_2galactose	Galactose; Fucose; Xylose	B, C, I
			0·47	Me xylose		
10	155	+70	0·48	2,3-Me_2galactose	Galactose; Xylose	B, I, P
			0·39	4-Me xylose; 3-Me xylose (*t*)		
11	40	+66	0·40	2,4-Me_2galactose; Me xylose (*t*)	Galactose; Xylose (*t*)	D, E, I, P
12	22	+11	0·29	Me xylose; Unknown sugar	Xylose; Galactose (*t*)	D, E, I
13	30	+70	0·28	Unknown sugar	Arabinose	D, E, I
			0·24	2-Me galactose	Galactose	
14	320	+84	0·23	2-Me galactose	Galactose	
15	150	+45	0·23	2-Me galactose		D, E
			0·17	Xylose		
			0·09	Galactose		

* *t* = trace. † B, C, D, and E = paper chromatography in solvents B, C, D, and E, respectively; I = paper ionophoresis; P = paper chromatography of the periodate-oxidised sugar.

used as eluants to give fifteen fractions. The annexed Table summarises the results of preliminary examination of the various fractions.

Fraction 1. The syrup crystallised to give 2,3,4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 90—91°, $[\alpha]_D$ +60° ⟶ +17° (equil.) (*c* 1·2), and the sugar was further characterised by conversion into 2,3,4-tri-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 55—56°.

Fraction 2. The syrup was fractionated on filter sheets with solvent D, to give fractions 2*a* (40 mg.) and 2*b* (61 mg.). Fraction 2*a*, $[\alpha]_D$ +20° (*c* 0·8), crystallised to give 2,3,4-tri-*O*-methyl-D-xylose, m. p. and mixed m. p. 90—91°. Fraction 2*b* had $[\alpha]_D$ −118° (*c* 0·75) and gave fucose and a trace of xylose on demethylation. Crystallisation from ethanol containing 1% of water furnished 2,3,4-tri-*O*-methyl-L-fucose monohydrate, m. p. and mixed m. p. 63—64°. The sugar also afforded an aniline derivative, m. p. 132—133° (James and Smith [22] give m. p. 133—134° for 2,3,4-tri-*O*-methyl-*N*-phenyl-L-fucosylamine).

Fraction 3. The complex mixture of sugars was partly fractionated on filter sheets with solvent B, giving fractions 3*a* (44 mg.) and 3*b* (96 mg.). Fraction 3*a* had $[\alpha]_D$ +116° (*c* 0·9) and chromatography in solvents A, B, C, and D and paper ionophoresis indicated that the main component was 2,3,4,6-tetra-*O*-methylgalactose and that only traces of tri-*O*-methylfucose and tri- and di-*O*-methylxylose were present. 2,3,4,6-Tetra-*O*-methyl-D-galactose was characterised by conversion into the aniline derivative, m. p. and mixed m. p. 189—190°. Chromatography of fraction 3*b*, $[\alpha]_D$ −40° (*c* 0·96), indicated the presence of 3,4-di-*O*-methylxylose, a di-*O*-methylfucose, and a trace of tetra-*O*-methylgalactose. A further component was revealed by paper ionophoresis and the major portion was further separated by ionophoresis on filter sheets into three sub-fractions. Chromatography and ionophoresis of fraction 3*b* (i) (15 mg.) showed a main component, R_G 0·80, which gave fucose on demethylation. Gas chromatography of the methyl glycosides on column *a* showed two main components, *T* 1·08 and 1·52. Fraction 3*b* (ii) (10 mg.), $[\alpha]_D$ +22° (*c* 1·0), was characterised as 3,4-di-*O*-methyl-D-xylose by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 67—68°. Fraction 3*b* (iii) (6 mg.) was chromatographically and ionophoretically indistinguishable from 3,5-di-*O*-methyl-L-arabinose, gave arabinose on demethylation, and furnished methyl glycosides having the relative retention times (*T* 1·06, 2·75) of methyl glycosides of 3,5-di-*O*-methyl-L-arabinose on column *a*.

Fraction 4. The syrup was separated by ionophoresis on filter sheets into fractions 4*a* (20 mg.) and 4*b* (62 mg.). Fraction 4*a* contained three components, probably a di-*O*-methylfucose (R_G 0·80) and 2,3- and 2,4-di-*O*-methylxylose (R_G 0·73, 0·69), and gave fucose and xylose on demethylation. Fraction 4*b*, $[\alpha]_D$ +21° (*c* 1·5), was chromatographically and ionophoretically homogeneous and was characterised as 3,4-di-*O*-methyl-D-xylose by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 67—68°.

Fraction 5. The syrup was converted into methyl glycosides which were examined by gas chromatography. Components having the retention times of methyl glycosides of 2,4,6-(major) and 2,3,4-tri-*O*-methyl-D-galactose (minor) (*T* 4·16 and 4·76, and 7·5 on column *b*, and 2·10 and 2·39, and 2·66 and 2·93 on column *c*) were recognised, but the complex mixture of glycosides from other sugars (probably including di-*O*-methylxylose) could not be identified with certainty.

Fraction 6. The syrup contained 2,3,4-tri-*O*-methyl-D-galactose as the main component and the sugar was characterised as the aniline derivative, m. p. and mixed m. p. 164—165°.

Fraction 8. The syrup, $[\alpha]_D$ +32° (*c* 1·0), contained a major component, M_G 0·38, which was purified by chromatography in solvent A. Chromatography of the periodate-oxidised sugar showed a series of products similar to those formed by 3-*O*-methylrhamnose. Gas chromatography of the methyl glycosides showed a main component (*T* 1·00 on column *c*) which was distinct from the methyl glycosides formed from 2-*O*-methyl-L-fucose.

Fraction 10. Gas chromatography of the derived methyl glycosides on column *c* indicated the presence of methyl glycosides of 4-*O*-methylxylose (*T* 1·10) and 2,3-di-*O*-methylgalactose (*T* 2·48, 3·23, 3·75, 4·24). The syrup was fractionated on filter sheets with solvent E, to give pure samples of 4-*O*-methyl-D-xylose (40 mg.) and 2,3-di-*O*-methyl-D-galactose (50 mg.), together with a mixture (45 mg.) of the two sugars. 4-*O*-Methyl-D-xylose crystallised when seeded, had m. p. and mixed m. p. 102—104° and $[\alpha]_D$ +11° (equil.) (*c* 2·0), and was further identified by an *X*-ray powder photograph. 2,3-Di-*O*-methyl-D-galactose, $[\alpha]_D$ +77° (*c* 0·75),

[22] James and Smith, *J.*, 1945, 746.

was characterised by conversion into the aniline derivative which was identified by m. p. 153—164° and mixed m. p. (with sample m. p. 152—153°) 152—154°, and by *X*-ray powder photograph.

Fraction 11. The syrup crystallised to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 97—98°, which was further characterised by conversion into the aniline derivative, m. p. and mixed m. p. 209—210°.

Fraction 14. The sugar crystallised from ethanol–water to give 2-*O*-methyl-D-galactose, m. p. and mixed m. p. 146—148°, and $[\alpha]_D$ +52° ⟶ +84° (equil.).

Fraction 15. The major portion of the syrup was fractionated on filter sheets with solvent D, to give 2-*O*-methyl-D-galactose (21 mg.), m. p. and mixed m. p. 147°, $[\alpha]_D$ +85° (equil.) (*c* 1·0), D-xylose (80 mg.), $[\alpha]_D$ +80° ⟶ +19° (equil.) (*c* 4·0), m. p. and mixed m. p. 144—145° [di-*O*-benzylidene dimethyl acetal, m. p. and mixed m. p. 210°], and D-galactose (29 mg.), $[\alpha]_D$ +80° (equil.) (*c* 1·3), m. p. and mixed m. p. 166° [mucic acid, m. p. and mixed m. p. 210°].

Examination of Hydrolysis Products from Methylated and Reduced Methylated Tragacanthic Acid.—Samples (100 mg.) of methylated and methylated reduced tragacanthic acid were hydrolysed and the hydrolysates were separated on filter sheets with solvent D into four fractions containing (i) tri- and di-*O*-methylpentoses and 6-deoxyhexoses, tetra- and tri-*O*-methylhexoses (R_G 0·93—0·60), (ii) *O*-methylpentoses and di-*O*-methylhexoses (R_G 0·60—0·25), (iii) *O*-methylhexose and unsubstituted sugars (R_G 0·25—0·10), and (iv) methylated acids (chromatographically immobile). The various fractions were converted into methyl glycosides which were examined by gas chromatography on columns *b* and *c*. In addition, the methylated acids from methylated tragacanthic acid were treated with methanolic hydrogen chloride, reduced with lithium aluminium hydride, and hydrolysed to give neutral sugars which were examined by paper chromatography and whose methyl glycosides were examined by gas chromatography on column *c*. The results indicated that 2,3,4-tri-, 2,3(and possibly 2,4)-di-, and 2-*O*-methylgalactose were formed only after reduction of hexuronic acid units.

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319. *Gum Tragacanth. Part II.*[1] *The Arabinogalactan.*

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Hydrolysis of the methylated derivative of the arabinogalactan from gum tragacanth furnishes 2,3,5-tri-, 2,3-, 2,5-, and 3,5-di-, 2- and 3-*O*-methyl-L-arabinose, L-arabinose, 2,4,6-tri-, 2,3- and 2,4-di-, and 2-*O*-methyl-D-galactose, D-galactose, 4-*O*-methyl-L-rhamnose, 2,3,4-tri- and 2,3-di-*O*-methyl-D-galacturonic acid, and traces of other sugars. Degraded polysaccharides have been prepared by mild acid hydrolysis and by degradation of the periodate-oxidised arabinogalactan, and the cleavage products from their methylated derivatives have been examined by chromatographic techniques.

In Part I [1] it was shown that the arabinogalactan component of gum tragacanth may be isolated conveniently by extraction of the gum with ethanol–water (7 : 3). This polysaccharide preparation was essentially homogeneous when chromatographed on diethylaminoethylcellulose and hydrolysis afforded small proportions of galacturonic acid and rhamnose as constituent sugars in addition to arabinose and galactose. In earlier studies on the polysaccharide James and Smith [2] showed that when gum tragacanth is methylated

[1] Part I, preceding paper.
[2] James and Smith, *J.*, 1945, 749.

the derivative of the arabinogalactan may be separated from that of the major polysaccharide component, tragacanthic acid. The following sugars were recognised as cleavage products from the methylated arabinogalactan, 2,3,5-tri- and 2,3-di-*O*-methyl-L-arabinose, L-arabinose, and an unidentified di-*O*-methyl-D-galactose. These results indicated that the polysaccharide was highly branched and, although strict evidence of homogeneity was not available, suggested that the structure was based on a core of D-galactose residues to which were attached highly ramified chains of L-arabinofuranose residues. This conclusion is supported by the results of the present investigation in which further structural details have been established.

Methylated arabinogalactan was prepared by etherification of the separated polysaccharide and by methylation of the water-soluble portion of the gum followed by separation of the methylated derivative. The methylated arabinogalactan was hydrolysed and the following sugars were characterised by the formation of crystalline derivatives: 2,3,5-tri-, 2,3-, 2,5- and 3,5-di-, 2- and 3-*O*-methyl-L-arabinose, L-arabinose, 2,4,6-tri-, 2,3- and 2,4-di-, and 2-*O*-methyl-D-galactose, D-galactose, 4-*O*-methyl-L-rhamnose, and 2,3,4-tri- and 2,3-di-*O*-methyl-D-galacturonic acid. In addition, evidence was obtained for the presence in the hydrolysate of an unknown di-*O*-methylgalactose and 3,4-di-*O*-methylrhamnose. These results show that the L-arabinose residues, presumably present in the furanose form only, are involved in almost all the possible combinations of 1,2-, 1,3- and 1,5-linkages, and that the D-galactose residues are present mainly as single or double branching points.

Under controlled conditions partial acid hydrolysis of the arabinogalactan resulted in selective cleavage of arabinose residues, and a degraded galactan was isolated showing that the polysaccharide contains a core of contiguous D-galactose residues. A preliminary examination of this material showed that the degraded polysaccharide was still branched but that 1,6-linkages predominated. Paper chromatography of the products of partial acid hydrolysis indicated that 6-*O*-galactosylgalactose was the disaccharide formed in greatest amount, but that the 1,3-linked isomer was also produced. Gas–liquid chromatography [3,4] of the mixture of methyl glycosides of methylated sugars formed on methanolysis of the methylated degraded galactan indicated the presence of methyl glycosides of 2,3,4-tri- (major component), 2,3,4,6-tetra-, 2,3,6- and 2,4,6-tri-, and 2,4-di-*O*-methyl-galactose.

Further information concerning the highly ramified outer chains of L-arabinofuranose residues in the polysaccharide and an indication of the way in which some of these chains are attached to the inner chains of D-galactopyranose residues was obtained by degrading the arabinogalactan in a stepwise manner by removing those sugar residues which were attacked by periodate in the procedure of Smith and his co-workers.[5] The periodate-oxidised arabinogalactan was reduced with potassium borohydride, and controlled hydrolysis with cold dilute acid then afforded degraded arabinogalactan A. Two further degradations furnished successively degraded arabinogalactans B and C. After each degradation the degraded polysaccharide was recovered by precipitation from aqueous solution and products of low molecular weight were isolated from the mother-liquors. Paper-chromatographic examination of the low-molecular-weight products, before and after acid hydrolysis, showed that glycerol was the main component of each of the mixtures and that only small amounts of glycosidically linked components were present. It follows that the degradations resulted mainly in gradual erosion of the outer layers of sugar residues in the polysaccharide. At each stage of the degradation the relative proportions of arabinose to galactose residues in the degraded polysaccharide decreased showing that arabinose residues were being removed preferentially.

Degraded arabinogalactans A and B were methylated and the relative proportions of

[3] Bishop and Cooper, *Canad. J. Chem.*, 1960, **38**, 388.
[4] Aspinall, *J.*, 1963, 1676.
[5] Goldstein, Hay, Lewis, and Smith, Amer. Chem. Soc. Meeting, Boston, April 1959, Abs. Papers, 3D.

the cleavage products from the methylated derivatives were assessed semiquantitatively, together with those from the methylated undegraded polysaccharide; this was done by comparing relative intensities of spots on paper chromatograms given by the sugars formed on hydrolysis and relative peak heights from the gas–liquid chromatography of the methyl glycosides formed on methanolysis. By using these two techniques it was possible to detect all the methyl ethers of [illegible] and to establish individual characterised as cleavage products from the original methylated arabinogalactan. Methylated degraded arabinogalactans A and B furnished qualitatively similar mixtures of sugars and in each case 2,3,5-tri-*O*-methylarabinose was a major cleavage product. These residues, which appeared as end groups in the degraded polysaccharides, arose from interior chains in the parent polysaccharide. Since the first two degradations resulted in the exposure of new arabinofuranose end groups in the degraded polysaccharides it is certain that the majority and probably that all the L-arabinose residues in the polysaccharide are present in the furanose form. New arabinofuranose end groups (III) would be exposed by the degradation of sequences of sugar residues as in (I) or (II) in which the sequence is attached to another sugar residue which is resistant to glycol cleavage.

(I) Araf 1 ⟶ 2 (or 3) Araf 1 Araf 1 ⟶ 2 (or 3) Araf 1

5
↑
Araf (II)

(III) Araf 1

Changes in the mode of linkage of most of the galactose residues on degradation of the arabinogalactan were not readily apparent, but the results of the above experiments showed that 2,3,4-tri-*O*-methylgalactose could be detected as a cleavage product from each of the methylated degraded polysaccharides but not of the methylated undegraded arabinogalactan. It follows that such 1,6-linked galactopyranose residues (V) arose from residues which carried side-chains invariably composed of arabinofuranose residues which were cleaved by periodate, *e.g.*, (IV). Since the arabinogalactan contains arabinofuranose residues in the outer chains and galactopyranose residues in the inner chains only, such a degradative sequence (IV ⟶ V) indicates the way in which some of the units of the two sugars are joined.

...6 Galp 1... ⟶ ...6 Galp 1... (V)

3
↑
R (IV) R = Araf 1... or Araf 1 ⟶ 5 Araf 1...

The present results establish the general structural character of the arabinogalactan from gum tragacanth. The polysaccharide is composed of interior chains of D-galactopyranose residues, in which the majority of units are mutually joined by 1,6-linkages, and a smaller proportion by 1,3-linkages. These basal chains carry highly ramified exterior chains of L-arabinofuranose residues which are mutually joined by 1,2-, 1,3-, and 1,5-linkages. The polysaccharide also contains small proportions of D-galacturonic acid and L-rhamnose residues, whose modes of linkage were indicated by methylation. In view of the chromatographic homogeneity of the polysaccharide on DEAE-cellulose it is probable that these sugars are integral, albeit minor, constituents of the arabinogalactan, but nothing is yet known of the way in which units of these sugars are incorporated into the general molecular structure. The polysaccharide is notable in containing a high ratio of arabinose to galactose residues, but these results show that the polysaccharide has many structural features in common with those from other plant gums.[6]

Experimental

The general experimental procedures were as described in Part I.

Preparation and Hydrolysis of Methylated Arabinogalactan.—Arabinogalactan (fraction A14,[1] 5 g.) was methylated with methyl sulphate and sodium hydroxide and partly methylated

[6] Smith and Montgomery, "Chemistry of Plant Gums and Mucilages," Reinhold Publ. Corp., New York, 1959.

polysaccharide (4 g.) was isolated by extraction with chloroform (Found: OMe, 34·0%). This product was combined with similar material isolated during the preparation of methylated tragacanthic acid [1] (combined wt., 6 g.), and further methylation with methyl iodide and silver oxide furnished methylated arabinogalactan (4·2 g.), $[\alpha]_D$ −97° (*c* 1·7 in $CHCl_3$) (Found: OMe, 39·5%).

Methylated arabinogalactan (3·1 g.) was suspended in 2*N*-hydrochloric acid (100 ml.) for two days at room temperature. After addition of methanol (100 ml.) solution was complete and the mixture was heated slowly to 100°, care being taken that no material was precipitated as methanol was removed by distillation. The mixture was diluted with water so that the resulting solution was made *N* with respect to acid, and the hydrolysis was continued on the boiling-water bath for a further 15 hr. (constant rotation). The cooled solution was neutralised with silver carbonate and filtered, precipitation of silver was completed with hydrogen sulphide, and the hydrolysate was shaken with Amberlite resin IR-45(OH), to remove acidic sugars, and concentrated to a syrup (2·93 g.), which was separated on cellulose (74 × 4 cm.), (i) light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3, later 1 : 1), saturated with water, (ii) butan-1-ol, half saturated with water, and (iii) water being used as eluants to give twelve fractions. A further fraction containing acidic sugars was eluted from the ion-exchange resin with *N*-formic acid.

Fraction 1. The chromatographically pure syrup (961 mg.), R_G 0·95 and $[\alpha]_D$ −40° (*c* 1·2), gave arabinose only on demethylation and was characterised as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. 135—136° and mixed m. p. (with sample, m. p. 134—136°) 134—136°.

Fraction 2. Chromatography of the syrup (181 mg.), $[\alpha]_D$ −20° (*c* 0·51), in solvents A, B, and C showed a complex mixture of sugars including 2,3,5-tri-, 2,5/3,5-, and 2,3-di-*O*-methylarabinose, di-*O*-methylrhamnose, and tri-*O*-methylgalactose, and demethylation of the syrup gave arabinose, galactose, and rhamnose. The syrup (170 mg.) was refractionated on cellulose (45 × 2 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (4 : 1), saturated with water, as eluant, to give fractions 2*a* (28 mg.), 2*b* (15 mg.), 2*c* (85 mg.), and 2*d* (24 mg.). Fraction 2*a*, R_G 0·95 and $[\alpha]_D$ −39° (*c* 1·4), was chromatographically pure 2,3,5-tri-*O*-methyl-L-arabinose. Chromatography of fraction 2*b*, $[\alpha]_D$ +34° (*c* 0·9) in solvents A, B, and C, and ionophoresis indicated 3,4-di-*O*-methylrhamnose (major component) and a mixture of 2,5- and 3,5-di-*O*-methylarabinose. Gas chromatography of the derived methyl glycosides on column *c* showed components having the retention times of methyl glycosides of 3,4-di-*O*-methylrhamnose (*T* 0·97) and 2,5-di-*O*-methylarabinose (*T* 2·02). Demethylation of fraction 2*b* gave rhamnose and arabinose. Chromatography and ionophoresis of fraction 2*c*, $[\alpha]_D$ −18°, showed 2,5- and 3,5-di-*O*-methylarabinose as major components with traces of 2,3-di-*O*-methylarabinose and 2,4,6-tri-*O*-methylgalactose. Chromatographically and ionophoretically pure samples of the two main components were obtained after ionophoresis on filter sheets. 2,5-Di-*O*-methyl-L-arabinose (45 mg.) was characterised by conversion into 2,5-di-*O*-methyl-L-arabonolactone, m. p. 59–60° (depressed on admixture with 3,5-di-*O*-methyl-L-arabonolactone) (Smith [7] gives m. p. 60° for 2,5-di-*O*-methyl-L-arabonolactone). 3,5-Di-*O*-methyl-L-arabinose (30 mg.) was characterised by conversion into 3,5-di-*O*-methyl-L-arabonolactone, m. p. and mixed m. p. 76—77°. Chromatography of fraction 2*d*, $[\alpha]_D$ +91° (*c* 1·2), in solvents A, B, and C showed 2,3-di-*O*-methylarabinose and 2,4,6-tri-*O*-methylgalactose, and demethylation gave arabinose and galactose. Gas chromatography of the derived methyl glycosides on column *c* showed main components having the retention times of methyl glycosides of 2,3-di-*O*-methylarabinose (*T* 0·62, 0·82, 0·93) and 2,4,6-tri-*O*-methylgalactose (*T* 2·00, 2·36).

Fraction 3. Chromatography of the syrup (282 mg.) {$[\alpha]_D$ +92° (*c* 1·0) in solvents A, B, and C} showed 2,3-di-*O*-methylarabinose and 2,4,6-tri-*O*-methylgalactose, and demethylation gave arabinose and galactose. The syrup (260 mg.) was fractionated on charcoal–Celite by gradient elution with water containing 5—25% of ethanol to give pure samples of both sugars and a small amount of a mixture (24 mg.). 2,3-Di-*O*-methyl-L-arabinose (98 mg.), $[\alpha]_D$ +101° (*c* 0·5), was characterised by conversion into 2,3-di-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 160—161°. 2,4,6-Tri-*O*-methyl-D-galactose (121 mg.), $[\alpha]_D$ +90° (*c* 1·2), was characterised by conversion into the aniline derivative, m. p. and mixed m. p. 172—173°.

Fraction 4. The syrup (64 mg.), R_G 0·57, 0·53, 0·42 and $[\alpha]_D$ +77° (*c* 0·8), was separated on

[7] Smith, *J.*, 1939, 744.

filter sheets with solvent C into fractions 4*a* (10 mg.; 2,4-di-*O*-methylgalactose), 4*b* (7 mg.; 2-*O*-methylarabinose), and 4*c* (40 mg.). Fraction 4*c*, $[\alpha]_D$ +20° (*c* 0·8), contained two components, R_G 0·54, 0·57, which were resolved by ionophoresis on filter sheets. Both sugars gave positive colour reactions with triphenyltetrazolium hydroxide (unsubstituted at C-2). The first sugar (19 mg.), R_G 0·54 and $[\alpha]_D$ +14° (*c* 0·4), crystallised from acetone–water, had m. p. 130—135°, and gave galactose on demethylation (Found: OMe, 26·0. Calc. for $C_8H_{16}O_6$: OMe, 29·8%). The second sugar, R_G 0·57 and $[\alpha]_D$ +10° (*c* 0·5), crystallised from acetone–water, gave rhamnose on demethylation, and had m. p. 113—115° and mixed m. p. with 4-*O*-methyl-L-rhamnose (m. p. 121—122°) 115—118°.

Fraction 5. Chromatography of the syrup (66 mg.), $[\alpha]_D$ +105° (*c* 1·4), showed 2,3-di-*O*-methylgalactose (R_G 0·46) as the main component with traces of 2,4-di-*O*-methylgalactose (R_G 0·42) and 2-*O*-methylarabinose (R_G 0·38). The main component (40 mg.) was separated from the two minor components (9 mg.) by chromatography on filter sheets in solvent C. The sugar, R_G 0·47 and $[\alpha]_D$ +81° (*c* 0·8), was converted into 2,3-di-*O*-methyl-*N*-phenyl-D-galactosylamine, which was identified by m. p. 137—139° and by *X*-ray powder photograph.

Fraction 6. Chromatography of the syrup (47 mg.), $[\alpha]_D$ +104° (*c* 1·1), showed 2-*O*-methylarabinose (R_G 0·38) with some 2,4-di-*O*-methylgalactose (R_G 0·42). Demethylation gave arabinose and a trace of galactose. The main portion (40 mg.) of the syrup was fractionated on a filter sheet in solvent B, to give 2-*O*-methyl-L-arabinose (34 mg.) which was characterised by conversion into the toluene-*p*-sulphonylhydrazone, m. p. 144—146° and mixed m. p. (with a sample of m. p. 147—148°) 145—148°.

Fraction 7. Chromatography of the syrup (81 mg.), $[\alpha]_D$ +91° (*c* 1·8), showed approximately equal amounts of 2-*O*-methylarabinose and 2,4-di-*O*-methylgalactose. Demethylation gave arabinose and galactose. Chromatography of the periodate oxidation products showed methoxymalondialdehyde (from 2-*O*-methylaldose) and unchanged 2,4-di-*O*-methylgalactose. 2,4-Di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 98—99°, gradually separated from the syrup.

Fraction 8. The sugar (121 mg.), R_G 0·41 and $[\alpha]_D$ +84° (*c* 1·2), which contained a trace of contaminating 2-*O*-methylarabinose (R_G 0·38), was recrystallised from acetone containing 1% of water to give 2,4-di-*O*-methyl-D-galactose monohydrate, m. p. and mixed m. p. 101—102°, $[\alpha]_D$ +120° ⟶ +85° (*c* 0·7) (aniline derivative, m. p. and mixed m. p. 210°).

Fraction 9. Chromatography of the syrup (40 mg.), $[\alpha]_D$ +83° (*c* 0·8), showed 2,4-di-*O*-methylgalactose (R_G 0·41) and 3-*O*-methylarabinose (R_G 0·32). Demethylation gave galactose and arabinose. Chromatography of the periodate oxidation products showed a main component (R_F 0·72, grey stain with aniline oxalate) and a trace of methoxymalondialdehyde (R_F 0·24) characteristic of the products from a 3-*O*-methylpentose, and unchanged 2,4-di-*O*-methyl galactose.

Fraction 10. The chromatographically pure sugar (288 mg.), R_G 0·32 and $[\alpha]_D$ +113° (*c* 2·2), gave arabinose on demethylation and was characterised as 3-*O*-methyl-L-arabinose by conversion into 3-*O*-methyl-L-arabonolactone, m. p. 76—77° and mixed m. p. (with sample, m. p. 75—77°) 75—76°.

Fraction 11. The sugar (256 mg.), R_G 0·29 and $[\alpha]_D$ +82° (*c* 2·0), which contained a trace of contaminating arabinose (R_G 0·21), recrystallised from ethanol–water to give 2-*O*-methyl-D-galactose, m. p. and mixed m. p. 145—146°, $[\alpha]_D$ +55° ⟶ +84° (*c* 1·9).

Fraction 12. Chromatography of the syrup (506 mg.), $[\alpha]_D$ +82° (*c* 2·1), showed arabinose (R_G 0·21), galactose (R_G 0·14), and methylated uronic acids (R_G 0·31 and 0·07). The syrup was separated on filter sheets in solvent E into fractions 12*a* (240 mg.), 12*b* (25 mg.), 12*c* (17 mg.), and 12*d* (52 mg.). Fraction 12*a* was L-arabinose, m. p. and mixed m. p. 158—159°, $[\alpha]_D$ +160° ⟶ +104° (*c* 1·4) (toluene-*p*-sulphonylhydrazone, m. p. and mixed m. p. 154—155°). Fraction 12*b* was D-galactose, m. p. and mixed m. p. 166—167°, $[\alpha]_D$ +140° ⟶ +80° (*c* 1·2) (mucic acid, m. p. and mixed m. p. 210—211°). Fractions 12*c* and 12*d* were combined with fractions 13*a* and 13*b* for further examination.

Fraction 13. The syrup (101 mg.; eluted from Amberlite resin IR-45) contained methylated uronic acids (R_G 0·31 and 0·07) and neutral sugars which had been retained by the resin. Neutral sugars were separated from the barium salts of the acids by extraction with boiling acetone. The acids were released after removal of barium ions on Amberlite resin IR-120(H), and fractionation on filter sheets in solvent E gave fractions 13*a* (13 mg.) and 13*b* (28 mg.). The combined fractions 12*c* and 13*a* were converted into methyl ester methyl glycosides,

reduced with lithium aluminium hydride in tetrahydrofuran, and hydrolysed to furnish a syrup (10 mg.), $[\alpha]_D$ +115° (*c* 1·0), R_G 0·72, which crystallised from ethanol to give 2,3,4-tri-*O*-methyl-D-galactose, m. p. and mixed m. p. 80—81°, $[\alpha]_D$ +147 ⟶ +120° (*c* 1·1). The combined fractions 12*d* and 13*b* similarly afforded 2,3-di-*O*-methyl-D-galactose (41 mg.), R_G 0·49, $[\alpha]_D$ +85° (*c* 0·9), which was characterised as the aniline derivative, identified by m. p. 133—135° and *X*-ray powder photograph.

Partial Acid Hydrolysis of Arabinogalactan (with R. N. FRASER and R. STIRLING).—Arabinogalactan (0·25 g.) was heated in 0·1N-hydrochloric acid (25 ml.) on the boiling-water bath. Samples were withdrawn at intervals, degraded polysaccharide was precipitated by the addition of ethanol (4 vol.), and the supernatant liquid was neutralised with Amberlite resin IR-45(OH) and concentrated. The soluble sugars and the hydrolysate from the degraded polysaccharide were each examined chromatographically. The results showed that arabinose was rapidly released together with small amounts of galactose and traces of rhamnose. After 22 hr. the degraded polysaccharide gave mainly galactose and only traces of arabinose on hydrolysis. Under the same conditions arabinogalactan (7 g.) furnished degraded galactan (1·1 g.). Degraded galactan (1·0 g.) was methylated with methyl sulphate and sodium hydroxide, to give partially methylated galactan (0·55 g.), which was further methylated with methyl iodide and silver oxide to give methylated galactan (*ca.* 200 mg.), $[\alpha]_D$ +26° (*c* 0·47 in $CHCl_3$) (Found: OMe, 45·5%). Chromatography of the hydrolysate from the methylated galactan in solvent A indicated tetra- (medium), tri- (strong), and di-*O*-methylgalactose (weak). Methylated galactan was heated with methanolic 4% hydrogen chloride in a sealed tube for 18 hr., the cooled solution was neutralised with silver carbonate, filtered, and concentrated, and the resulting syrup was examined by gas chromatography on columns *b* and *c*. Table 1 shows the relative retention times (*T*) of methyl glycosides of methylated sugars which were indicated.

TABLE 1.

Examination of methanolysis products from methylated degraded galactan by gas chromatography.

Sugar	Approx. relative propns.	Relative retention times (*T*) of methyl glycosides: Column *b*	Column *c*
2,3,4,6-Tetra-*O*-methylgalactose	++	1·80	1·53, (1·61)
2,3,4-Tri-*O*-methylgalactose	+++	7·5	2·62, 2·89
2,3,6-Tri-*O*-methylgalactose	+	3·25, 3·93 (4·30), (4·75)	(1·60) (2·11) 2·22, (2·48)
2,4,6-Tri-*O*-methylgalactose	++	4·19, (4·75)	(2·11), 2·38
2,4-Di-*O*-methylgalactose	+		3·74, 4·44

Arabinogalactan (0·1 g.) was heated in 0·5N-sulphuric acid (10 ml.) on the boiling-water bath for 1 hr. The cooled solution was neutralised with barium carbonate, filtered, and concentrated. The resulting syrup was eluted from charcoal–Celite (1 : 1; 10 g.) with water (500 ml.) and with water containing 10% of ethanol (250 ml.). Chromatography of the latter eluant showed disaccharides with the mobilities of 6- (major product) and 3-*O*-β-D-galactopyranosyl-D-galactose, and a third disaccharide (possibly a 1,4-linked galactobiose in trace amounts).

Degraded Arabinogalactans A, B, and C.—Arabinogalactan (17 g.) was oxidised with 0·1M-sodium metaperiodate (2·445 l.) for 30 hr. (uptake of reagent was constant and corresponded to the consumption of 0·58 mole of reagent with the release of 0·04 mole of titratible acid per sugar residue), and the excess of reagent was destroyed by addition of ethylene glycol (17 g.). The solution was passed through Amberlite resin IR-120(H) to remove sodium ions, and iodic acid was neutralised with barium hydroxide. The filtered solution was treated with potassium borohydride (14 g.) for 24 hr., the excess of which was then destroyed. Potassium ions were removed by Amberlite resin IR-120(H), and the solution was concentrated, methanol being added to facilitate removal of boric acid as methyl borate. The concentrated solution was made N with respect to sulphuric acid and left at room temperature for 3 hr. The resulting solution was neutralised with barium hydroxide, barium sulphate was removed at the centrifuge, and degraded arabinogalactan A (7 g.), $[\alpha]_D$ −21° (*c* 1·3), was precipitated by the addition of ethanol (4 vol.). Hydrolysis of degraded arabinogalactan A gave arabinose and galactose in the approximate proportions of 2·5 : 1, and traces of rhamnose. Concentration of the mother-liquor from the precipitation gave a syrup which was shown by chromatography to contain

glycerol as the main component together with two non-reducing substances, $R_{arabinose}$ 1·10 and 0·93 (traces only). These minor components were probably glycerol glycosides of arabinose and galactose since hydrolysis of the syrup gave arabinose and a trace of galactose.

Degraded arabinogalactan A (3·5 g.) was similarly degraded by oxidation with sodium metaperiodate (0·62 mole of reagent was consumed with the release of 0·06 mole of titratable acid per sugar residue), reduction with potassium borohydride, and hydrolysis with cold N-sulphuric acid, to furnish degraded arabinogalactan B (1·5 g.), $[\alpha]_D$ +15° (*c* 0·9), hydrolysis of which gave arabinose and galactose in the approximate proportions of 1·5 : 1, and a trace of rhamnose. In a further degradation degraded arabinogalactan B (0·75 g.) (0·61 mole of periodate were consumed per sugar residue) afforded degraded arabinogalactan C (0·35 g.) $[\alpha]_D$ +48° (*c* 2·1), hydrolysis of which gave arabinose and galactose in approximately equimolecular proportions.

Analysis of Cleavage Products from Methylated Arabinogalactan and Methylated Arabinogalactans A and B.—Methylated arabinogalactan was heated with methanolic 4% hydrogen chloride in a sealed tube for 10 hr., the cooled solution was neutralised with silver carbonate, filtered, and concentrated, and the resulting syrup was examined by gas chromatography on columns *b* and *c*. Table 2 shows the relative retention times (*T*) of methyl glycosides of methylated sugars which had been characterised previously by the formation of crystalline derivatives.

TABLE 2.

Examination of methanolysis products from methylated arabinogalactan by gas chromatography.

Sugar	Relative retention times (*T*) of methyl glycosides: Column *b*	Column *c*
2,3,5-Tri-*O*-methylarabinose	0·56, 0·72	0·46, (0·59)
2,3-Di-*O*-methylarabinose	1·56, 1·78 (1·89)	0·64, (0·84) 0·96
2,5-Di-*O*-methylarabinose	1·89, 3·46	0·69, 1·02
3,5-Di-*O*-methylarabinose	1·06, 2·54	(0·59) (0·84)
2-*O*-Methylarabinose	6·10	(1·04) (1·47)
3-*O*-Methylarabinose	4·34, 6·96	1·27, (1·47), 1·59
2,4,6-Tri-*O*-methylgalactose	4·15, 4·74	2·08, 2·35
2,3-Di-*O*-methylgalactose		2·47, 3·15
2,4-Di-*O*-methylgalactose		(3·71) (4·20) (3·71) 4·41
2,3-Di-*O*-methylgalacturonic acid *	5·30	2·19

* Present as methyl ester. Figures in parentheses indicate *T* values of components which were incompletely resolved.

TABLE 3.

Examination of cleavage products from methylated arabinogalactans.

Sugar	Methylated arabinogalactan	Methylated degraded arabinogalactan A	Methylated degraded arabinogalactan B
2,3,5-Tri-*O*-methylarabinose	++++	+++	+++
2,3-Di-*O*-methylarabinose	++	++	++
2,5-Di-*O*-methylarabinose	+	+	+
3,5-Di-*O*-methylarabinose	+	+	+
2-*O*-Methylarabinose	++	+	tr.
3-*O*-Methylarabinose	+++	+	+
arabinose	+++	+	tr.
2,4,6-Tri-*O*-methylgalactose	++	++	++
2,3,4-Tri-*O*-methylgalactose		++	++
2,4-Di-*O*-methylgalactose	++	++	++
2-*O*-Methylgalactose	++	++	++
galactose	Trace	Trace	Trace

Methylated degraded arabinogalactans A (Found: OMe, 40·3%) and B (Found: OMe, 41·0%) were prepared by treatment of the parent polysaccharides (*ca.* 0·5 g.) with methyl sulphate and sodium hydroxide, and methyl iodide and silver oxide.

The cleavage products from methylated degraded arabinogalactans were similarly examined by (*a*) paper chromatography of the hydrolysates in solvents A, B, C, and D, and (*b*) gas

chromatography of the methyl glycosides formed on methanolysis on columns *b* and *c*. Table 3 indicates the relative proportions of the cleavage products from the various methylated arabinogalactans.

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IDENTIFICATION BY PAPER CHROMATOGRAPHY OF SWELLING AGENTS IN FOODSTUFFS

Testing for swelling agents or thickeners in pure form presents no difficulties. A compilation by Letzig (1) lists a number of precipitating reactions which permit distinguishing between the several thickeners in simple manner. However, these compounds seldom exist in such pure form that the use of such precipitating reactions, presented in synoptic form, permits non-ambiguous conclusions. Even the occurrence of two different swelling agents simultaneously can make a clear identification difficult; when there are more than two, this is true even more so. However, not only a mixture of these substances complicates the tests but also the presence of other compounds among which the proteins are predominant. Letzig's reagents are mainly protein precipitants so that precipitation will always occur in the presence of proteins. It is easy, of course, to determine the presence of proteins but this does not yet allow us to decide whether, in the absence of proteins, precipitation will have been due to thickeners. Prior to testing, proteins must therefore be removed and Wyler (2) has already described a possibility for this, specifically for meat products. This suggested removal of proteins with the aid of Carrez precipitation is so thorough, however, that not only are many swelling agents precipitated for which we must thus test prior to precipitation but the possibility of identifying those still remaining in solution is also restricted to a very few. A method of complete removal of proteins without interfering with subsequent testing and identification of swelling agents is not yet known so that the Letzig reagents in practice unfortunately have only a limited importance. As seductive as such tests are in their simplicity, they alone very rarely furnish non-ambiguous

indications of the kind of the swelling agents present. There is a possibility of precipitating the proteins with alcohol which can subsequently be easily evaporated (e.g. in vacuum) but this has the disadvantage that the proteins are not always completely eliminated and that some thickeners, specifically carob-seed flour, are also precipitated.

These disadvantages raise the question of different testing methods and Letzig already indicates in the report cited that the application of paper chromatography promises success. Since most thickeners are constituted by carbohydrates, no difficulties exist from the point of view of paper chromatography; it is merely necessary to convert the test substance into a form suitable for paper-chromatographic testing. It may happen, however, that with several swelling agents present, a non-ambiguous conclusion on the kind of the individual substances is not possible since a number of thickeners have a similar composition and/or furnish similar cleavage products after hydrolysis. In that case, however, a combination with the precipitating reagents of Letzig would permit us to continue further since the decision will lie between the few still possible thickeners. Whereas the proteins do not interfere with such paper-chromatographic tests, we must first test for the presence of carbohydrates not originating from swelling agents. Admixtures of simple sugars easily determinted with the aid of paper chromatography, can generally be removed by extracation with 50-% alcohol which does not affect the swelling agents. It is sufficient to let the substance stand with the alcohol for several hours under occasional shaking. The extract and/or the filtrate can serve to test for sugar (3) whereas the thickeners can be determined from the residue.

Hydrolysis of Swelling Agents

Since thickeners do not directly respond to paper chromatography, it is necessary to convert them to a form which is accessible to this method of testing. Among the possibilities of hydrolytic cleavage, that using enzymes is excluded and,

since carbohydrates are involved, acid hydrolysis is indicated primarily in order to prevent decomposition of cleavage products. The following conditions have been shown to be suitable and have been steadily employed for some time:

10 g of pure swelling agent and/or test substance are diluted with 50 ml of water and 50 ml of a 10-% sulphuric acid solution. If the mixture is too thick so that the retort may burst under direct heating, it should be placed in a hot water bath for a short time. When fluid, the solution is boiled in a reflux condenser where duration is a function of the kind of the probable thickeners since each substance is hydrolyzed at different rates of speed. If no indication of the type of swelling agent exists, the solution is kept at boiling temperature for three hours. It can then be directly chromatographed. However, it is preferable to concentrate if there is only a small amount of test substance; sulphuric acid also interferes somewhat, specifically with uronic acids, so that it is preferable to remove the former. For this purpose, 30 g barium hydroxide (with 8 mol water) are dissolved in 100 ml of water under heating and dripped, under constant shaking, through a filter into the hot hydrolytic solution until the latter reaches a p_H of 7. With these generally known conditions for removal of the sulphuric acid, it is easy to adjust the p_H-value as above by spotting Merck indicator paper. The precipitated barium sulphate can be rapidly filtered out, after settling, through a standard filter. Since only small amounts are required, it is sufficient if a part of the filtrate is processed further. This part is concentrated under vacuum to 50% and thus reaches again the starting concentration. If only small amounts of swelling agent are available, these can be further concentrated as desired. This solution is now suitable for paper chromatography. It is not at all necessary to keep within the quantitative relations indicated above and they can all be reduced correspondingly which is of value when only small amounts of test substance are available.

Paper-Chromatographic Conditions

Paper chromatography always takes place under the conditions already described earlier (3 & 4). The fluxing agent is n-butanol/pyridine/water (3:2:1.5) and staining is done with phtaleic acid/aniline and/or naphthoresorcinol/trichloroacetic acid. The last-named staining for non-reducing sugars plays a subordinate role in this connection. The chromatograms thus obtained with the ascending method are shown in the figure at the end of the article. The parallel obtained test solutions permit non-ambiguous identification so that even closely adjacent spots cannot be confused with each other which may easily occur when working exclusively with R_f-values.

Evaluation

Fig. 1 shows two chromatograms of some frequently occurring swelling agents. It will be clearly seen that, after hydrolysis of carob-seed flour, only the spots of galactose and mannose appear. Although mannose is nearly equal in R_f-value to mannose, it can be distinguished from the reddish arabinose by its brown color tone but these colors cannot be recognized in a black-and-white photo. With agar-agar, only galactose is clearly seen whereas xylose is manifested only as a weak spot. Gum arabic shows galactose in addition to arabinose and rhamnose. Although rhamnose is recognized only vaguely, it is characteristic since it is only infrequently found in thickeners. In the sample examined by us, there occurs further a spot immediately below the starting point which we were never able to identify. Pectin shows mainly the spot of uronic acid which can be recognized from the typical light-brown color after staining with phthalate/aniline as well as by staining with naphthoresorcinol/trichloroacetate when it turns slightly blue after some time (cf. 3 for details). We know that this involves galacturonic acid which paper chromatography cannot demonstrate since sufficient separation from glucuronic acid does not take place. The R_f-values of the two uronic acids do differ slightly and we might therefore assume that differentiation would be possible after sufficient time. Here we must take into account that the R_f-values of the two

acids are to a considerable extent a function of the concentration of these acids and of the kind of an eventually existing cation. Something similar has already been shown for some other organic acids (5). The variations so caused are greater, however, than the difference between the two R_f-values. In addition to the spot of galacturonic acid, a weak spot of glucose also occurs with pectin. It must be remembered further that a duration of hydrolysis of three hours must be strictly kept, in contrast to most other swelling agents. Whereas a duration of hydrolysis of one to two hours generally produces complete cleavage in the latter, galacturonic acid in pectin shows only weakly after one hour but increases with continued hydrolysis. Tragacanth gum shows the spots of galactose, arabinose and xylose. Comparison with the test solutions which were run parallel on the left chromatogram, makes possible rapid identification of the individual spots. The first test solution on the left shows the upper spot as galactose and the lower spot as mannose; the second test solution indicates the upper spot as arabinose and the lower as rhamnose. We already mentioned that arabinose and mannose cannot be differentiated in the figure since it is not possible to see the differences in color. The chromatogram makes it impossible to mix up the red color of arabinose with the brown color of mannose. The last test solution shows galacturonic acid at the top and, below this, xylose.

The chromatogram on the right of the figure shows "tylose" (methyl cellulose) on the left side which shows as expected the spot of glucose and below this non-hydrolyzed methylated glucoses. The occurrence of these methylated glucoses was in several preparations and appears to be characteristic for most of the tylose preparations. It is possible, however, that this is a function of manufacture and that these spots may therefore be absent some times. The adjacent cellulose glycolate also shows the spot of glucose and, in the upper part of the chromatogram, two and occasionally three unidentified spots which always have a characteristic semilunar form. Alginate exhibits only the spot of a uronic acid. The adjacent test solutions show in the

first case from top to bottom the spots of lactose, maltose and glucose whereas glucuronic acid is shown on the left.

When several thickeners are present, identification on the basis of the chromatogram can become difficult. It then seems preferable to make identification less through the spots that do show but initially through those absent. The absence of uronic acid permits a definite conclusion that neither pectin nor alginate exist. However, we believe sufficient concentration of the hydrolyzed solution must be made in order to avoid that a uronic acid does not show by reason of excessive dilution. The absence of galactose excludes the presence of carob-seed flour, agar-agar, gum arabic and tragacanth. Absence of glucose means that neither tylose nor cellulose glycolate, generally also no pectin and obviously no swelling starch can be present. Lack of mannose would exclude carob-seed flour and that of arabinose would eliminate gum arabic and tragacanth. Here we must take into account, however, that the presence of one of the two last-named spots does not permit a conclusion on the absence of the other since a possible superposition may exist which does not allow this.

With this method of selection, most of the known swelling agents can easily be excluded so that we do obtain in general a non-ambiguous picture of the composition of the mixture of swelling agents. Where in complicated cases several interpretations are possible, we can turn to the precipitating reactions Letzig mentioned above for clarification of any remaining doubts, in spite of the interferences indicated. When direct interpretation is not possible, the method of selection has proved itself also for the Letzig reactions. In most cases, however, paper chromatography should be sufficient or at least give decisive indications. As in most investigative methods, a certain intuitive judgment will develop with practice.

As described, such testing for swelling agents is obviously restricted only to those whose composition includes carbohydrates. Although these represent the greater number, this does not allow us to neglect others, e.g., gelatine. Testing for such substances

by specific paper chromatography would evidently have to be carried out from other viewpoints.

Conclusion

1. The precipitating reactions in testing for swelling agents are excellent for pure compounds but rarely furnish non-ambiguous findings for mixtures and in the presence of other substances, e.g. proteins.

2. Since most of the swelling agents are constituted by carbohydrates, paper chromatography of the sugars and/or sucroid compounds offers the possibility of identifying swelling agents by hydrolytic cleavage.

3. Hydrolysis is made with sulphuric acid and boiling at an average duration of 3 hours. After removal of sulphuric acid by barium hydroxide and concentration of the clear filtrate under vacuum, the test is made with a method described in an earlier communication.

4. Evaluation is best made by a method of selection in which all swelling agents are excluded which cannot exist due to the absence of a spot. However, it is necessary to take into account the ratios of concentration.

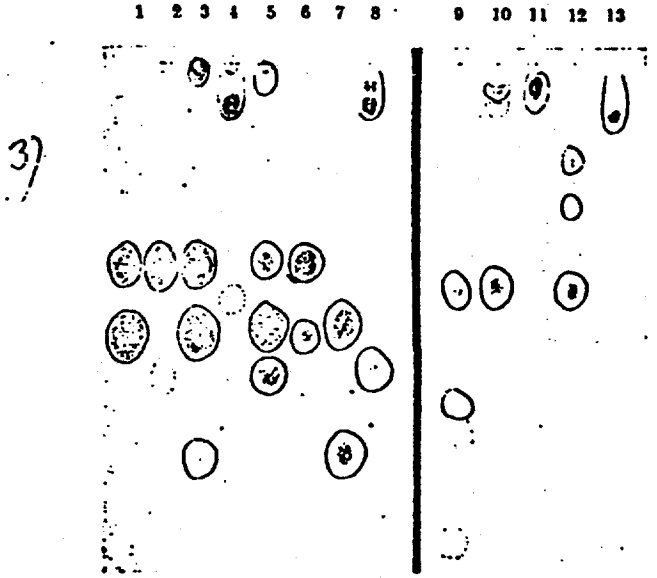

Abb. 1. *Mit Schwefelsäure hydrolysierte Quellstoffe und entsprechende Testlösungen.* Fließmittel: n-Butanol/Pyridin/Wasser (3:2:1,5), Färbung: Phthalsäure/Anilin. *Linkes Chromatogramm:* (von links) *1* Johannisbrotkernmehl, *2* Agar-Agar, *3* Gummi-arabicum, *4* Pektin, *5* Traganth. Testlösungen: *6* Galaktose + Mannose, *7* Arabinose + Rhamnose, *8* Galakturonsäure + Xylose. *Rechtes Chromatogramm:* (von links) *9* Tylose, *10* Celluloseglykolat, *11* Alginat. Testlösungen: *12* Lactose + Maltose + Glucose, *13* Glucuronsäure

Fig. 1 - Chromatogram of swelling agents hydrolyzed with sulphuric acid and the resulting test solutions. Fluxing agent: n-butanol/pyridine/water (3:2:1.5). Staining: Phthaleic acid/aniline. Left side: 1= carob-seed flour; 2 = agar-agar; 3 = gum arabic;

4 = pectin; 5 = tragacanth; test solutions: 6 = galactose + mannose; 7 = arabinose + rhamnose; 8 = galacturonic acid + xylose. Right side: 9 = tylose; 10 = cellulose glycolate; 11 = alginate; test solutions: 12 = lactose + maltose + glucose; 13 = glucuronic acid.

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ALLERGY (ASTHMA) TO INGESTED GUM TRAGACANTH

A Case Report*

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ALLERGIES due to gums are being reported with increasing frequency. The various gums have a widespread use in daily life. Gum acacia given intravenously was tried out as a blood substitute, but had to be rejected on account of the frequency of sensitizations following its use.[1] Karaya gum is used by printers as a dryer and has been reported as an occupational hazard.[9] Individuals working in factories where gums are inhaled have developed asthma and perennial rhinitis.[2, 3, 4, 6, 7] Other sources of contact have been wave sets, toothpastes, dental adhesive, candy fillers, ice cream, salad dressings, emulsions, pills, lotions, and catharties.[8] Dermatitis from contact and gastrointestinal distress from ingestion have been recorded.[5, 7]

In the various reports on gums, acacia, karaya gum, and tragacanth have been the three most common allergens in the order listed. Osgood[10] reported using five gums in routine testing. These were acacia, ghatti, karaya, tragacanth, and quince seed. Of 100 cases routinely tested in his allergy clinic, 98 reacted to one or more gums. Some of the authors reporting cases of sensitivity to gums have had success with the passive transfer test.[4, 6, 7]

CASE REPORT

A white male (M.D.), aged 33 years, was first seen in our clinic in 1940 with a history of sneezing and rhinorrhea from April through October. Aspirin and coal-tar drugs were said to have caused urticaria and rhinorrhea three to four hours after ingestion. The patient had had excessive contact with gum tragacanth from 1924 to 1927 when he worked in a candy manufacturing establishment. Intradermal skin tests on admission were positive to trees, grasses, and ragweed pollen extracts. The patient was treated perennially with moderate relief of symptoms. He took treatment poorly and had frequent constitutional reactions.

On April 22, 1946, the patient was given 50 mg. tablets of pyribenzamine† three times a day. This resulted in marked alleviation of sneezing and rhinorrhea, but toward the end of the week he developed heavy breathing and omitted the drug. On May 6, 1946, he was given pyribenzamine placebos.† After taking four tablets there was a return of sneezing and he stopped the medication. On May 20, 1946, he was again given the placebo pills, and four hours after taking one pill he developed asthma. The following day he took another placebo, and in four hours he had asthma and urticaria. He received no further medication until June 17, when he was given 50 mg. of benadryl which relieved his rhinorrhea but caused marked drowsiness. On September 9, he was again given pyribenzamine. He took 50 mg. on arising and 50 mg. after lunch; three to four hours after the last pill he developed severe asthma but no urticaria. Concomitantly there was about 80 per cent relief from rhinitis. The asthma persisted for thirty-six hours.

One 50 mg. placebo tablet was dissolved in 10 c.c. of saline. An intracutaneous test with 0.05 c.c. of the solution showed a moderate local reaction. Eight minutes after the test there was generalized urticaria. The placebo tablet contained talcum, lactose, mineral oil, magnesium stearate, and gum tragacanth. Scratch tests with these materials were all negative except for gum tragacanth which gave a moderate reaction. Scratch test with 1 per cent karaya gum also gave a moderate reaction.

*From The Department of Allergy, The Roosevelt Hospital, New York City, N. Y.
†Furnished by Ciba Co.

On Oct. 14, 1946, blood was taken for passive transfer, and the following dilution and neutralization tests were carried out.

It can be seen from the above tables that gum tragacanth was the dominant antigen among the three gums tested.

TABLE I. DILUTION TESTS*

SERUM "MD"	SITES TESTED WITH 0.1 C.C. OF		
	1 PER CENT ACACIA	1 PER CENT KARAYA	1 PER CENT TRAGACANTH
Conc.	slight	slight	moderate
1:10	negative	negative	moderate
1:100	negative	negative	slight

*Three sites of 0.1 c.c. of "MD" serum in each of the noted dilutions were made on the back of a nonallergic test subject. Each series of serum dilutions were tested with one of the three gums and with the results recorded above.

TABLE II. NEUTRALIZATION TESTS*

SERUM "MD" MIXED WITH EQUAL PARTS OF	SITES TESTED WITH 0.1 C.C. OF		
	1 PER CENT ACACIA	1 PER CENT KARAYA	1 PER CENT TRAGACANTH
1 per cent acacia	negative	very slight	moderate
1 per cent karaya	negative	negative	moderate
1 per cent tragacanth	negative	negative	very slight

*Equal parts of "MD" serum and 1 per cent solution of each of the three gums were separately mixed, and three sites of 0.1 c.c. of each mixture were made in the back of a nonallergic test subject. Each series of sites was tested with one of the gums forty-eight hours later with the results as recorded above.

COMMENT

A case of asthma and urticaria due to gum tragacanth is presented. The antigen was taken orally as a part of a tablet. The patient had several other allergic manifestations, but asthma had never been noted until the treatment with pyribenzamine containing gum tragacanth as an excipient was given. Each 50 mg. tablet of pyribenzamine and the placebo contains 0.005 Gm. of gum tragacanth. Apparently very small amounts of this material are capable of causing severe symptoms. It is also of interest that the combination of pyribenzamine and gum tragacanth relieved the symptoms of sneezing and rhinorrhea but not those of asthma.

In view of the wide usage of the various gums, these allergens should be used more often in routine testing. Although cross reactions between the gums may occur, some patients react to only one or two.[10] It is also suggested that before ascribing sensitivity reactions to the main ingredient of a medication, the other constituents also should be investigated.

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Identification of Stabilizing Agents

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EXTENSIVE use of a variety of polysaccharides as stabilizing or thickening agents in foods has created a need for analytical methods for the identification of commercially available materials and for their detection and quantitative estimation in food products. A number of stabilizers, which are used or have been suggested for use in foods, are listed and their chemical nature is indicated in Table I. Throughout this report the term "gums" is used in referring to all of these materials. Gelatin is included because it has many uses similar to those of the polysaccharides.

As the initial step in a project concerned with the development of the required analytical procedures, methods for the qualitative identification of a number of gums have been studied. Several procedures for the identification of gums are found in the literature. A method described by Jacobs and Jaffe (*15*) classifies the gums on the basis of physical characteristics or appearance of precipitates and thus requires considerable experience on the part of the analyst. Their outline does not include pectic substances, alginates, methylcellulose, carboxymethylcellulose, gum ghatti, starch, or gelatin. It also has the disadvantage of using an unstable reagent (Millon's) which must be freshly prepared each day. A method developed by Cannon and ad-

The present work was undertaken to develop a method for the identification of stabilizing and thickening agents used in food products. The materials studied were pectin, de-esterified pectin, algin, Irish moss, gum tragacanth, gum karaya, locust bean gum, starch, agar, gum arabic, gum ghatti, carboxymethylcellulose, methylcellulose, and gelatin. A proposed identification scheme is based on precipitation reactions with calcium chloride, sodium hydroxide, barium hydroxide, and lead acetate. In addition, reactions of the stabilizing agents with a cationic soap, ammonium sulfate, mercuric nitrate, papain, and gelatin are listed. The proposed scheme should be useful for identification of unknown stabilizing agents. A number of the reactions reported might be employed for the identification of individual stabilizing agents in mixtures of these materials or isolated from foods.

the Association of Official Agricultural Chemists (*5, 6*) does not provide for the identification of pectic substances, alginates, methylcellulose, carboxymethylcellulose, locust bean (carob) gum, or gum ghatti. Bryant (*4*) has described a procedure for distinguishing between pectin and certain gums, but it does not provide for positive identification of the gums. A number of other publications dealing with characteristic properties of these polysaccharides have been summarized by Mantell (*16*), but a systematic procedure is still needed for their identification. Such a procedure would be useful for the identification of products used as thickeners or stabilizers in foods, drugs, and cosmetics and ultimately for the identification of polysaccharides isolated from these materials.

In the present investigation, the manner in which the gums disperse in water after being wetted with alcohol has been a valuable index to the identity of unknown samples. Their solubility properties are summarized in Table II. Use has also been made of the fact that many of the polysaccharides occur as salts of complex organic acids (Table I). The acidic properties may be due to the presence of uronic acid groups, as in gum arabic, or to the unesterified portion of sulfuric acid molecules esterified with the polysaccharide. When mineral acids are added to mucous solutions or dispersions of these salts, the effective concentrations of the polysaccharide anions are decreased. Thus, although the complex anions may yield insoluble salts with heavy metal cations, most are not precipitated from acid solutions. The amount the pH must be raised in order to precipitate the heavy metal salts—e.g., barium, mercury, or lead—is frequently characteristic of the individual polysaccharide.

Table I. Source and Chemical Nature of Materials Commonly Used as Thickening Agents in Foods

Material	Source	Principal Components	References
Pectic substances	Fruits	Galacturonic acid (occurs as methyl ester)	(*18*)
Algin (sodium alginate)	Seaweeds	Mannuronic acid (Na salt)	(*16*)
Irish moss	Seaweeds	Galactose, galactose 4-sulfate (K and Ca salts)	(*18*)
Agar	Seaweeds	Galactose (D- and L-), galactose 6-sulfate (Ca and Mg salts)	(*18*)
Tragacanth	Plant gum	L-Fucose, D-xylose, galacturonic acid, L-arabinose, D-galactose	(*18*)
Methylcellulose	Modified cellulose	Methyl-D-glucose	(*18*)
Starch	Plants	D-Glucose	(*18*)
Carboxymethylcellulose	Modified cellulose	Carboxymethyl-D-glucose	(*18*)
Locust bean gum (carob gum)	Seed endosperm	Mannose and galactose	(*18*)
Guar gum	Seed endosperm	Mannose and galactose	(*20*)
Karaya	Plant gum	Galactose, acetic acid, galacturonic acid, rhamnose, tagatose	(*14*)
Arabic (acacia)	Plant gum	D-Glucuronic acid, D-galactose, L-arabinose, rhamnose (mixed Ca, Mg, and K salts)	(*18*)
Ghatti	Plant gum	L-Arabinose, galactose, galacturonic acid (Ca salt)	(*16*)
Gelatin	Modified protein	Amino acids	

EXPERIMENTAL

During the present investigation 0.5 to 1.0% aqueous dispersions of the polysaccharides were used for the tests. Aliquots of from 3 to 5 ml. were treated with varying concentrations of the reagents which it was hoped would give characteristic precipitation reactions. Initially the reagents used were those for which Jacobs and Jaffe (*15*) have described reactions with several polysaccharides. Subsequently a number of other reagents were used.

Table II. Dispersion in Water of Gums, Wetted with Alcohol

Gum	Manner of Dispersal in Water
Pectic acid	Insoluble
Pectate (Na, K, or NH_4 salts)	Forms either clear or turbid solution on heating
Pectate (Ca salts)	Insoluble
Pectin	Swells in cold water and dissolves on heating
Alginate	Dissolves slowly in cold water or quickly on heating to form viscous solution
Irish moss	Dissolves slowly in cold water, rapidly on heating to form viscous solution
Agar	Swells in cold water, dissolves on heating, gels on cooling
Tragacanth	Swells to form viscous dispersion in cold or hot water, but does not form true solution
Methylcellulose	Dissolves slowly in cold water but becomes cloudy or gels on heating
Starch	Disperses on heating
Carboxymethylcellulose	Dissolves slowly in cold water, rapidly on heating, giving clear viscous solution with some fine fibrous suspended material
Locust (carob)	Forms viscous suspension but not a true solution
Karaya	Forms viscous suspension. Insoluble particles settle on standing
Arabic (acacia)	Dissolves in cold water to form a clear only slightly viscous solution
Ghatti	Dissolves to form almost clear solution but some insoluble material may remain as fine suspension
Gelatin	Swells in cold water and dissolves on heating

Reactions which were found useful for characterizing the gums are summarized in Tables III and IV. Only those materials having anionic components, such as alginates, or potential anionic components, such as pectin, give pronounced reactions with cationic soap (Table III). As in the case of precipitates with heavy metals, the precipitates with the cationic soap quickly disperse on acidification of the medium. Ammonium sulfate is of interest, in that it gives pronounced precipitation tests with several of the gums but not with alginates, pectin, tragacanth, karaya, arabic, or ghatti, each of which probably contains uronic acid components. The reactions with Stokes's acid mercuric nitrate illustrate the effects of low pH on precipitation of heavy metal salts of the polysaccharide acids. An excess of the reagent makes the solutions strongly acidic and thus the weakly dissociated acids redisperse. Alginic acid and pectic acid are insoluble and thus are not dissolved by excess Stokes reagent. Papain and gelatin give pronounced precipitation reactions only with those gums having anionic components. These precipitates are found only if the

Table III. Precipitation Reactions of Polysaccharide Gums and Gelatin

Gum	1 Vol. 1% Solution of Cationic Soap[a]	0.5 Vol. Saturated Ammonium Sulfate	Diluted[b] Stokes's Acid Mercuric Nitrate Added Dropwise	1 Vol. 2% Papain (6)[c]	1 Vol. 2% Gelatin[c]	4 Vol. 95% C_2H_5OH + 2 – 3 Drops Saturated NaCl
De-esterified pectin	Fine opaque precipitate	Gelatinous translucent precipitate	Gels (almost opaque). Insoluble in excess reagent	Precipitate	Precipitate	Gelatinous precipitate, gels (1 vol.)
Alginate	Fine opaque precipitate	Nil	Gels (almost opaque). Insoluble in excess reagent	Precipitate	Precipitate	Gelatinous precipitate (1 vol.) becomes stringy with 4 vol. alcohol
Pectin	Flocculent precipitate	Nil	Forms almost opaque gel which dissolves in excess reagent	Cloudy	No definite effect	Transparent gelatinous precipitate. Gels (1 vol.)
Irish moss	Stringy or flocculent precipitate	Gelatinous precipitate or gel	Transparent gel. Redispersed by excess reagent	Precipitate	Precipitate	Stringy precipitate
Agar	Gelatinous precipitate	Flocculent precipitate	Turbid or cloudy	Cloudy	Precipitate	Fine flocculent precipitate
Tragacanth	Fine opaque precipitate	Nil	Flocculent precipitate. Dissolves in excess reagent	Precipitate	Precipitate	Voluminous precipitate, jellylike
Methylcellulose	Nil	Precipitate	Nil	Nil	Nil	Nil
Starch	Nil	Precipitate	Nil	Nil	Nil	Opaque flocculent precipitate
Carboxymethylcellulose	Gelatinous clotted precipitate	Gelatinous precipitate	Precipitate dissolves in excess reagent	Precipitate	Precipitate	Voluminous clotted precipitate
Locust	Nil	Precipitate (voluminous)	No pronounced effect	No pronounced effect	Nil	Voluminous opaque stringy precipitate, forms clot
Karaya	Flocculent precipitate	Nil	Flocculent precipitate dissolves in excess reagent	Precipitate	Precipitate	Flocculent precipitate, discrete particles
Arabic (acacia)	Precipitate (very fine)	Nil	Flocculent precipitate dissolves in excess reagent	Precipitate	Precipitate	Fine opaque nonsettling precipitate
Ghatti	Fine precipitate	Nil	Nil	Nil	Precipitate	Fine precipitate, nonsettling (2–3 vol.)
Gelatin	Precipitate in alkaline medium (22)	Precipitate	Nil	Nil	Nil	Finely flocculent precipitate, coagulates

[a] Rodalon (alkyl dimethyl benzyl ammonium chloride), Fairfield Laboratories, Plainfield, N. J.
[b] Mercury dissolved in twice its weight of concentrated nitric acid and diluted to 100 times its volume with distilled water.
[c] Precipitates with papain and gelatin are observed only in weakly acidic medium and most exhibit properties of coacervates rather than true precipitates.

pH of the mixture is below the isoelectric point of the protein and it is possible that they would be more correctly called coacervates. They are usually dispersed by a few drops of mineral acid or of dilute ammonium hydroxide. The characteristic manner in which some of the gums are precipitated by alcohol may also be of value in their identification.

The reactions described in Table IV form the basis of a proposed procedure for the systematic identification of the gums.

REAGENTS

Calcium chloride ($CaCl_2$), 3% solution (weight/volume).
Ammonium hydroxide, 3.0 *N* solution.
Sodium hydroxide, 3.0 *N* solution.
Barium hydroxide, saturated solution stored in a bottle equipped with a siphon and a soda-lime tube.
Basic lead acetate, 20% suspension (weight/volume). Heat to boiling, cool, and use supernatant solution.
Hydrochloric acid (or other mineral acid), 3.0 *N* solution.
Methylene blue, 0.1% aqueous solution.
Tincture of iodine (U.S.P., *14*).
Iodine–potassium iodide stock solution, containing 0.5% iodine and 1.0% potassium iodide. Iodine–potassium iodide test solution, consisting of stock solution diluted 1 to 5.
Cupric sulfate ($CuSO_4 \cdot 5H_2O$), 15% solution (weight/volume).
Borax ($Na_2B_4O_7 \cdot 10H_2O$), 4% solution.
Ruthenium red test solution (*3*).
Picric acid, saturated aqueous solution.

IDENTIFICATION PROCEDURE

Wet a 0.25- to 0.5-gram sample of the material to be identified with 1 to 2 ml. of 95% alcohol and add 50 ml. of distilled water. Suspend the solid material in the water by shaking or stirring. Heat the suspension, with frequent shaking, on a hot plate or over a burner. If the sample dissolves, discontinue heating immediately; otherwise hold at 85° to 95° C. for 15 minutes.

Group A. I. Treat a 3- to 5-ml. aliquot of the solution with 0.2 volume of 0.25 *M* calcium chloride. A gelatinous precipitate or gel indicates alginates or de-esterified pectin.

If no reaction is apparent with calcium chloride alone, add 1 volume of 3 *N* ammonium hydroxide to the calcium chloride treated sample. Slow formation of a gel or gelatinous precipitate indicates pectin.

II. If either test in A I was positive, mix a fresh 3- to 5-ml. aliquot of sample with 1 volume of 3.0 *N* sodium hydroxide. Observe the reaction and then heat the mixture in a boiling water bath for 10 minutes.

Immediate formation, in the cold, of a gelatinous or flocculent precipitate indicates either pectin or de-esterified pectin. No precipitate indicates aliginates. All three mixtures become yellow on heating, but the precipitates with pectic substances do not dissolve.

Group B. If the material is not an alginate or a pectic substance, carry out the following tests.

I. Mix a 3- to 5- ml. aliquot of the sample with 0.1 volume of saturated barium hydroxide. Observe in the cold and heat in boiling water bath for 10 minutes.

Formation in the cold of a nonsettling, almost opaque, gelatinous precipitate indicates Irish moss. Carry out confirmatory test for Irish moss.

A small amount of flocculent precipitate or cloudiness in the cold and a definite lemon yellow color on heating identify gum tragacanth.

Color changing during heating to yellow, then to green, and finally to gray indicates agar. Carry out confirmatory test for agar.

If the mixture becomes cloudy or forms a gel on heating, but becomes clear on cooling, methylcellulose is indicated. Carry out confirmatory test for methylcellulose.

An opaque flocculent precipitate which may tend to redisperse on heating and reprecipitate on cooling indicates starch. Carry out confirmatory test for starch.

Precipitates which disappear when the barium hydroxide is thoroughly mixed with the sample may be disregarded at this point.

II. If the material has not been identified, mix a fresh 3- to 5-ml. aliquot of sample with 1 volume of saturated barium hydroxide. Observe whether there is an immediate precipitation and examine again after standing 5 minutes at room temperature.

Table IV. Precipitation Reactions Used as Basis of Proposed Procedure for Identification of Gums

(Reactions used as identification tests within heavy lines)

Gum	1/5 Vol. 2.5% $CaCl_2$	1/5 Vol. 2.5% $CaCl_2$ + 1/5 Vol. 3 *N* NH_4OH	1 Vol. 3 *N* NaOH	1/10 Vol. Saturated $Ba(OH)_2$ Cold	1/10 Vol. Saturated $Ba(OH)_2$ Heated	1 Vol. Saturated $Ba(OH)_2$	1/5 Vol. Basic Lead Acetate	Basic Lead Acetate + 1/5 Vol. 3*N* NH_4OH	Confirmatory Tests
De-esterified pectin	Gelatinous ppt. or gel		Gelatinous or flocculent ppt., yellow color on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Gels	As for lead acetate alone	Gelatinous ppt. when acidified with mineral acids
Alginate	Gelatinous ppt. or gel		Clear yellow soln. on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Gels	As for lead acetate alone	Gelatinous ppt. when acidified with mineral acids
Pectin		Gelatinous ppt. forms slowly	Gelatinous or flocculent ppt., yellow color on heating	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	As for 1/10 vol.	Translucent gel	As for lead acetate alone	
Irish moss	Some samples may give flocculent ppt.			Gelatinous ppt. or gel, almost opaque	Ppt. may flocculate with prolonged heating	Gelatinous ppt. or gel	Gelatinous ppt. or gel	As for lead acetate alone	Forms blue fibrous ppt. with aqueous methylene blue
Tragacanth				May be small amount of ppt.	Lemon yellow color	Flocculent ppt.	Flocculent ppt.	As for lead acetate alone	
Agar				Nil	Becomes yellow then green and gray	Nil	Flocculent ppt.	Gels	Gives blue or black stain with tincture of iodine
Methylcellulose				Nil	Becomes turbid or forms gel; becomes clear on cooling	Nil	Nil	Gels	Aqueous dispersions are not precipitated by alcohol, but form gel or become cloudy when heated
Starch				Opaque flocculent ppt.	May redisperse	Flocculent ppt.	Flocculent ppt.	Very heavy flocculent or gelatinous ppt.	Blue stain with I_2-KI reagent
Carboxymethylcellulose				Ppt. dissolves on shaking	Nil	Flocculent ppt.	Gels	As for lead acetate alone	Forms clotted ppt. with $CuSO_4$
Locust[a]						Clotted opaque ppt.	Opaque gel	As for lead acetate alone	Gels with 1/5 vol. 4% borax
Karaya						Flocculent ppt., forms slowly	Flocculent ppt.	As for lead acetate alone	Swells and stains pink with ruthenium red test solution
Arabic (acacia)							Voluminous opaque ppt.	As for lead acetate alone	Readily soluble in water at room temperature
Ghatti							May be small amount of flocculent ppt.	Voluminous opaque ppt.	Fine ppt. with 4 vol. of alcohol. Arabic also gives fine ppt.
Gelatin									Gives fine yellow ppt. when added to saturated picric acid

[a] Locust bean gum and guar gum give identical reactions and cannot be distinguished on basis of these tests.

Table V. Samples Examined by Proposed Identification Procedure

Gum	Type	No. of Samples
Sodium pectate	Powdered	1
Pectic acid	Granular	1
Pectin	Powdered	2
Sodium alginate	Powdered	5
Irish moss	Powdered	2
Tragacanth	Powdered	1
Agar	Shredded	1
	Granular	3
Methylcellulose	Fibrous	2
Starch	Whole wheat flour	1
	Tapioca flour	1
	Soluble starch	3
	Cornstarch	1
	Amioca (amylopectin)[a]	1
	Clear jel[a]	1
	Clear-Flo-II (sodium salt of starch acid ester)[a]	1
	Dry-Flo (starch ester)[a]	1
	Vulca (starch ether)[a]	1
	Meljel[a]	1
	Nu-film (starch acid ester)[a]	1
Sodium carboxymethylcellulose	Powdered	1
Locust bean (carob)	Powdered	7
Guar	Powdered	1
Karaya	Powdered	1
Arabic (acacia)	Powdered	3
	Lump	1
Ghatti	Powdered	1
	Lump	1
Gelatin	Granular	2

[a] Products of National Starch Products, New York, N. Y.

A voluminous opaque, stringy precipitate which tends to form a clot indicates locust bean gum. This precipitate may appear flocculent if the mixture is shaken vigorously. Carry out confirmatory test for locust bean gum.

A voluminous opaque flocculent precipitate which forms immediately indicates carboxymethylcellulose. Carry out confirmatory test for carboxymethylcellulose.

An opaque flocculent precipitate which forms slowly and is not voluminous indicates gum karaya. Carry out confirmatory test for karaya.

Group C. If the sample has not been identified it may be gum arabic, gum ghatti, or gelatin.

I. Mix a fresh 3- to 5-ml. aliquot of sample with 1 ml. of basic lead acetate solution. Immediate formation of a voluminous opaque precipitate indicates gum arabic.

If there was only a small amount of flocculent precipitate, or no precipitate, with the basic lead acetate add 1 ml. of 3.0 *N* ammonium hydroxide to the lead-containing sample. A voluminous opaque flocculent precipitate indicates gum ghatti. If there is no precipitate, the sample probably is gelatin. Carry out confirmatory test for gelatin.

CONFIRMATORY TESTS

Alginates and De-esterified Pectins. Add 0.2 volume of 3 *N* hydrochloric acid (or other mineral acid) to 3 to 5 ml. of the sample. A gelatinous precipitate confirms alginates or de-esterified pectin.

Irish Moss. Add 2 to 3 drops of 0.5% methylene blue in water to 1 ml. of the sample solution. Precipitation of purple-stained fibers confirms Irish moss.

Methylcellulose. Mix 5 ml. of sample with 25 ml. of 95% alcohol and 2 to 3 drops of saturated sodium chloride. No precipitate confirms methylcellulose.

Agar. Precipitate gum from 5 ml. of sample with alcohol and stain with tincture of iodine (*3*). Starch is also stained blue by this reagent.

Starch. Add 1 to 2 drops of the iodine potassium iodide test solution to 1 ml. of sample. A blue or purple color confirms starch. Some samples of gum tragacanth may give a faint blue test here.

Carboxymethylcellulose. Add 2 ml. of 1.0 *M* cupric sulfate to 3 to 5 ml. of sample solution. An opaque, slightly bluish, clotted precipitate confirms carboxymethylcellulose.

Locust Bean Gum. Add 1 ml. of 4% borax to 3 to 5 ml. of gum solution. If mixture gelatinizes, locust bean gum is confirmed. Guar gum also forms a gel here.

Karaya. Precipitate gum from 5 ml. of solution with alcohol and stain with ruthenium red (*3*). If sample swells considerably and is stained pink, karaya is confirmed.

Gelatin. Add 2 to 3 drops of gum solution to 2 ml. of saturated picric acid. A fine yellow precipitate confirms gelatin.

DISCUSSION

The proposed procedure has been tested with the gums listed in Table V. It was possible to identify the modified starches as starch products, because the solubility of all these materials is decreased by barium hydroxide and all give positive tests with the iodine–potassium iodide reagent. It is possible that not all the thickening agents or gums employed in food products at the present time have been included in this study. Cherry gum and quince seed gum, for example, have been suggested in the literature for use in foods. However, the proposed procedure includes all the gums that were available during the investigation.

The proposed scheme for identification of stabilizing and thickening agents is applicable only when they have not been mixed with other materials. To identify thickening agents in foods by this method it would first be necessary to separate them from the foods, but separation techniques might alter the reaction characteristics of the gums. Much work has been done on methods for the separation and detection of gums in particular foods, such as mayonnaise and French dressing (*2, 7, 10, 11*), soft curd cheese (*1, 8, 10, 11, 13, 19*), tomato products (*10, 13*), starchy foods (*21*), cacao products (*13, 17, 23*), ice cream and frozen desserts (*9–11*), canned chicken (*10*), and meat products (*12*). Additional references to methods for separating gums from foods may be found in reviews by Jacobs and Jaffe (*15*) and Mantell (*16*). The emphasis in most procedures has been on detection of the gums without identification. However, Wyler (*24*) has outlined methods for the detection and identification of locust (carob) bean gum, methylcellulose, carboxymethylcellulose, starch, pectin, and alginate in meat products. Thus identification tests proposed in the present paper may be useful for the identification of gums separated from foods by methods already described in the literature. However, it is probable that many special techniques will be required for the analyses of particular combinations of foods and thickeners. A great deal more work must be done before it will be possible to identify all of the gums in the various foods in which they may be used.

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COMMENTS ON THE MECHANISM OF THE FETOTOXIC EFFECT OF TRAGACANTH

(Über den Mechanismus der fetalschädigenden Wirkung von Traganth)

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A. Introduction:

Not all the substances, whose possible teratogenic effect must be tested, are soluble in water, physiological salt solution and Ringer's solution. Hence, they must be applied, in the form of suspensions or emulsions according to their mode of utilization, orally or parenterally. Natural substances, beside synthetic products like carboxymethyl cellulose, are used as suspensions in manufacturing these preparations. That is why the influence of suspensions involving natural substances was tested in comparison with distilled water and Ringer's solution as to gestation in mice. This was done especially since WILLIAMSON and Collaborators (1963), as well as CARTER (1965), proved in their tests conducted on chick embryos that inert substances, like sand, ground glass, colloidal Al or talc, produced malformations with the same frequency as definitely teratogenic substances.

B. Material and Methods:

1. Animal Species Used:

Mice of the NMRI-breed were used for the tests (animals from closed random breeding; Breeder Dr. Haakh, until late 1964: Federal Research Institution for Virus Diseases in Animals, since 1965: Firma Ivanovas, Kissleg, Allgäu). Upon reception, all the mice received a protective inoculation of active vaccine virus against ectromelia at the Industrial Hygiene Pharmacological Institute of the BASF (Zeller and Reckzeh, 1965).

2. Type of the Substances Tested and of the Substance Specimens:

Table 1 contains a survey of the substances tested and substance specimens, and reveals their origins.

Table 1: Survey of the substances tested

Bezeichnung	Chargen-nummer	Herkunft	
Aqua destillata	--		
Ringerlösung	—		
Gummi arabicum	G 1012	E. Merck, AG	Darmstadt
Agar	51581	E. Merck, AG	Darmstadt
Carrageen	--	F. Schulze u. Co	Mannheim
Guar	M 175	Meyhall Chemical AG	Kreuzlingen (Schwe
Talkum	T 61754	E. Merck, AG	Darmstadt
persischer Traganth Nr. 0	--	E. Merck, AG	Darmstadt
persischer Traganth Nr. 1	434209	E. Merck, AG	Darmstadt
persischer Traganth Nr. 2	387011	E. Merck, AG	Darmstadt
persischer Traganth Nr. 3	263868	E. Merck, AG	Darmstadt
persischer Traganth Nr. 4	277520	E. Merck, AG	Darmstadt
persischer Traganth Nr. 5	419414	E. Merck, AG	Darmstadt
persischer Traganth Nr. 6	8021 65	Imhoff u. Stahl, GmbH	Mannheim
persischer Traganth Nr. 7	B 420,5165	Riedel de Haen, AG	Seelze-Hannover
indischer Traganth Nr. 1	14009,64	Imhoff u. Stahl, GmbH	Mannheim
indischer Traganth Nr. 2	562 65	Imhoff u. Stahl, GmbH	Mannheim

The terms "Persian tragacanth" designate the officinal tragacanths from the Near East. These are mucilages obtained mainly from Astragalus gummifer Labillardière and from related asiatic astragal species, all of them belonging to the Leguminosae family (TSCHIRSCH, 1912; BERGER, 1964). In previous time, this astragal mucus, called also "true tragacanth", was extended with "Indian tragacanth", and still today Indian tragacanth is often used as a substitute for the more expensive pure tragacanth.

Indian tragacanth, called also "false tragacanth", Karaya- or Sterculia-rubber, is obtained by scraping the bark of the trunk of Sterculia ureaus Roxb., a tree widely distributed in Hither India (Hindustan). Unlike true tragacanth, Sterculia-rubber contains no starch, possesses no adhesive power, and swells much more rapidly in water.

3. Nutrient Media:

Merck-Standard I-nutrient agar and Merck-Standard-nutrient broth were used in obtaining the bacteriological nutrient media. The various differential nutrient media were prepared according to the prescriptions indicated by HALLMANN ("Bakteriologische Nährböden" - Bacteriological Nutrient Media, Thieme-Verlag, Stuttgart, 1953).

4. Methode Used in Testing the Teratogenic Effect:

In teratogenic tests, the fertilization periods should be indicated as accurately as possible. Hence, in our Institute, when teratogenic tests are conducted on mice, several 100 pubescent female mice are placed together each time for 2 hours with the males (bucks) (5 females with one male), once a week from 10 to 12:00 AM. When the cohabitation is terminated, each animal exhibiting a vaginal plug is isolated. In the case of these "plug mices", the pairing day is called the 1st day of gestation (FROHBERG and OETTEL, 1964).

On the 19th day of gestation, thus one day before the normal littering day, all the treated or untreated mice are killed, and the number of the implantation places, of the intact and resorbed fetuses, as well as the number of the dead but externally uninjured fetuses, are determined. The implantation places, which can be recognized by the "metrial glands", which can be identified as quite perfused yellowish nodules on the mesometrium insertion of the uterine excrescences, and which are not occupied by living or artificially dead fetuses or fetal resorptions, characterize the number of the miscarried embryos.

The body length and weight of the fetuses are determined, then their malformations are examined macroscopically. This examination is conducted systematically from head to tail. Subsequently, the fetuses are fixed in alcohol; the soft parts are clarified in caustic potash and, in order to evaluate the individual bones, the skeletal system is dyed with alizarin red S by a modified Dawson method. These clarified and colored fetuses are kept in 100% glycerine DAB 6 (FROHBERG and OETTEL, 1966).

In order to be able to make a statement regarding the possible embryotoxic effect of a product in the case of the animal species used for the tests, one must possess an accurate knowledge of the spontaneous values. Hence investigations of the littering size, of the rates of fetal resorption and of malformation, were conducted on 413 pregnant and untreated NMRI mice.

In the uteri of 414 untreated animals, 4293 implantation places were found, that is 10.4 on an average per mother animal (See Table 2).

Table 2: Spontaneous values in 414 NMRI control-mice. The mother animals were caused to die on the 19th day of gestation.
a. Implantations b. Fetuses c. Alive d. Malformed e. Dead
f. Resorptions g. Miscarriages h. Total number i. Average per animal

	a. Implantationen	b. Feten	c.	d.	e.	f. Resorptionen	g. Aborte
		lebend	mißgebildet	runts	tot		
Gesamtzahl (h)	4293	3918	60	18	20	343	12
Durchschnitt pro Tier (i)	10,4	9,5	0,14	0,04	0,05	0,83	0,03

From the 4293 implanted embryos, 343 had died or had been resorbed. Thus the average quota of fetal resorption per animal was 0.83- One must bear in mind, however, that fetal resorptions were found only in 199 of the 414 control mice, and also one resorption in 120 animals, 2 resorptions in 44 mice, and more than 2 resorptions in 35 animals. One mouse, in each case, had even 6 or 8 spontaneous resorptions. On the 11th day of gestation, in a control animal, all the 12 fetuses were expelled as miscarriages.

For the most part, the mice had 9 - 13 fetuses per litter, and the 3918 living fetuses of the 414 control animals exhibited on an average a length of 2.2 cm and a body weight of 1.2 g. Eighteen living fetuses of the altogether 14 mother animals, must be regarded, with a body weight of only 0.6 - 0.8 g, as "congenital runts". In 18 mothers, altogether 20 dead, nonmalformed, "normally" developed fetuses were found in the uterus.

Figure 3: Spontaneous malformations in 3918 fetuses from 414 NMRI-control-mice. The mother animals were caused to die on the 19th day of gestation.
a. Type of malformation b. Number of malformed fetuses c. Frequency
d. Cleft palates e. Thoracic vertebral body hyplasty g. Aplasia of the vertebrae and ribs h. Malformations of the ribs i. Out of 50 mother animals.
f. execephaly. j. microcephaly k. micronathy

a. Art der Mißbildung		b. Zahl der mißgebildeten Feten	c. Häufigkeit (%)
d.	Gaumenspalten	40	1,02
f.	Exencephalie	6	0,15
j	Mikrocephalie	2	0,05
k	Mikrognathie	1	0,03
e	Brustwirbelkörperhypoplasie	4	0,10
g	Wirbel- und Rippenaplasie	1	0,03
h.	Rippenmißbildungen	6	0,15
		60	1,53
	i von 50 Muttertieren		

Out of the 3918 living fetuses, 60 (1.53%), which originated from 50 (12%) of the 414 mother animals, exhibited spontaneous malformations (Table 3), and also 40 fetuses (1%) had cleft palates. Thus they presented the most frequent spontaneous malformation in our mice-breed. Four fetuses had execephaly, 2 fetuses had microcephaly, 1 fetus exhibited a micrognathy, and in 4 fetuses, hypoplasiae or aplasiae of some thoracic vertebrae were detected. Six fetuses had malformations of the ribs, and in one stunted animal, all the thoracic vertabrae, the ribs, and most of the lumber vertebrae were missing.

C. Results:

1. Testing the Fetotoxic Effect of Different Suspensions:

From the 11th to the 15th day of pregnancy, gravid mother-mice received daily 0.2 ml per animal of the suspension preparations (mentioned in Table 4) by intraperitoneal injection. The individual results are shown in Table 4.

Table 4: Effects of suspensions on the pregnancy of NMRI mice 5 x 0.2 ml/animal intraperitoneally as aqueous suspension from the 11th to the 15th day of gestation (The mother animals were caused to die on the 19th day of gestation)

a. Substance b. Number of mother animals c. Total number per group with miscarried fetuses d. Implantations e. Total number per group f. Average per mother animal g. Resorptions h. % referred to the total number of implantations i. Miscarriages j. Fetuses k. Alive l. Weight m. Length n. Dead o. Malformed p. Type of malformation q. Distilled water r. Ringer's solution I. Cleft palate 2. Umbilical hernia 3. Malformation of the ribs 4. Aplasia of the Os interparietale.

a. Substanz	b. Muttertierzahl		d. Implantationen		g. Resorptionen		i. Aborte		j. Feten							
									k. lebend				n. tot	o. mißgebildet		
	c. ges.	mit mißgeb. Feten	ges.	ø	h. ges.	%	h. ges.	%	h. ges.	ø	l. Gewicht g ø	m. Länge cm ø	ges.	ges.	%	p. Art der Mißbildung
q. Aqua dest.	21	1	225	10,7	24	10,7	0	0	200	9,5	1,2	2,2	1	1	0,4	1 - 1
r. Ringerlösung	5	0	54	10,8	4	7,4	0	0	50	10,0	1,3	2,5	0	0	0	-
Gummi arabicum (1%)	8	1	92	11,5	3	3,3	0	0	88	11,0	1,3	2,2	1	1	1,1	1 - 2
Agar (1%)	9	2	94	10,4	9	9,6	4	4,3	81	9,0	1,2	2,2	0	4	4,3	3 - 1 1 - 3
Carrageen (1%)	9	1	92	10,2	9	9,8	0	0	82	9,1	1,2	2,2	1	1	1,1	1 - 1
Guar (1%)	11	0	113	10,3	42	37,2	9	8,0	52	4,7	1,1	2,1	10	0	0	-
Guar (1%) 5 × 0,1	8	0	72	9,0	12	16,7	2	2,8	55	6,9	1,1	2,1	3	0	0	-
Talkum (1%)	9	1	87	9,7	11	12,6	0	0	76	8,4	1,2	2,3	0	1	1,1	1 - 2
Traganth Nr. 0 (1%)	8	0	82	10,3	44	53,7	38	46,3	0	0	—	—	0	0	0	-
Gummi arabicum (10%)	18	5	202	11,2	57	28,2	0	0	145	8,1	1,2	2,3	0	6	3,0	3 - 1 3 - 4
Talkum (10%)	14	1	128	9,1	50	39,1	0	0	72	5,1	1,2	2,2	6	2	1,6	2 - 1

ges. = Gesamtzahl pro Gruppe, ø = Durchschnitt pro Muttertier, % = bezogen auf Gesamtimplantationszahl. 1 = Gaumenspalte 2 = Umbilicalhernie, 3 = Rippenmißbildung, 4 = Aplasie des Os interparietale.

After a 5 time intraperitoneal injection from the 11th to the 15th day of gestation of each time 0.2 ml per animal of distilled water, Ringer's solution, 1% aquaous gum arabic, agar and carrageen mucilage or of a 1% aqueous talc suspension, there was no disturbance of the pregnancy. After the injection of corresponding amounts of a 1% aqueous guar mucilage, one of the 11 mother mice died intercurrently. Forty-two fetuses were resorbed, 9 were expelled as miscarriages, and 10 were dead at the time of the killing of the mother animals

on the 19th day of gestation. In doses of 5 times 0.1 ml/animal, a 1% guar mucilage no longer influenced practically the fetal development.

After the 5 time intraperitoneal injection of each time 0.2 ml of a 1% aqueous mucilage of a commercially obtained Near East DAB 6 tragacanth, surprisingly all the fetuses were resorbed or were expelled as miscarriages. This ~~embryo~~feto-toxic effect of the 1% aqueous tragacanth mucilage investigated was considerably stronger than that of a 10% aqeous gum arabic mucilage or of a 10% aqueous talc suspension.

2. Embryotoxic Effect of Persion Tragacanth:

Since the ~~embryo~~feto-toxic effect (observed in the first test) of the investigated tragacanth specimen stood in contrast with all the other suspensions tested, and in order to exclude experimental errors or an accidental contamination of this specimen by unknown active substances, which could have caused the embryotoxic effect, 5 other charges (No. 1 - 5) of the DAB 6-tragacanth (of Persion origin) provided by the Merck Company and used in Test 1, as well as in each case one specimen of Persion tragacanth of the Imhoff & Stahl Company (No. 6) and of the Riedel de Haen Company (No. 7) were introduced into the investigations.

In these tests the animals were injected in each case with 0.2 ml of the 1% mucilages in distilled water only on the 11th and 12th day of gestation. Furthermore, some mice were treated on the 14th and 15th day of gestation with three different Persion tragacanth specimens in the corresponding way. The individual results are given in Figure 1.

Figure 1: Embryotoxic effect of Persion tragacanth for NMRI mice 2 x 0.2 ml/animal intraperitoneally as 1% aqeous mucilage. ▬ Resorptions, ▭ Miscarriages, ▭ Dead fetuses, ▭ Alive fetuses.

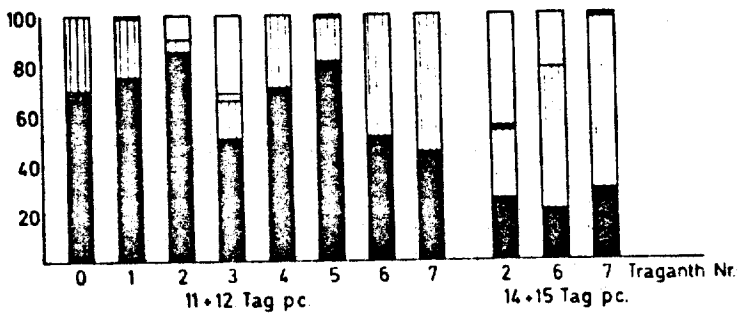

Abb. 1. Embryotoxische Wirkung persischen Traganths für NMRI-Mäuse 2 × 0,2 ml Tier i.p. als 1%iger wäßriger Schleim. ▬ Resorptionen, ▭ Aborte, ▭ [illegible] Feten, ▭ lebende Feten

Five hundred and twenty eight of the 818 fetuses of the 87 mother mice, into which each time 0.2 ml/animal had been injected intraperitoneally on the 11th and 12th day of gestation, were resorbed, and 255 were expelled as miscarriages. After injection on the 14th and 15th day of pregnancy, 12 of the 160 fetuses of the 15 mother animals were dead, 40 resorbed, and 82 were expelled as miscarriages.

The miscarriages, externally recognizable through vaginal bleedings, occurred in most cases 2 to 3 days after the treatment. While animals were being killed during vaginal bleeding, dead fetus residues were found in the uteri.

Thus all the tested charges of Persian tragacanth exerted a considerable embryotoxic effect.

Since already an only two-time intraperitoneal injection of 0.2 ml each time of a 1% tragacanth mucilage of Persian origin, had a highly ~~embryo~~feto toxic effect, a Persian tragacanth specimen of the three supplying Companies was injected into gravid mice only one intraperitoneally. The injections were conducted on the 11th, 12th or 14th day of gestation. See the individual results in Table 5.

Table 5: ~~Embryo~~feto toxic effect of Persian tragacanth for NMRI mice 1 x 0.2ml/animal intraperitoneally as 1% aqueous mucilage (The mother animals were caused to die on the 19th day of gestation)

a. Day of application percent b. Number of mother animals c. Total number per group d. with born fetuses e. Implantations f. Average per mother animal g. Resorptions h. % referred to the total number of implantatinns i. Miscarriages j. Fetuses k. Alive l. Weight m. Length n. Dead o. Malformed p. Type of malformation I = cleft palate 2 = Kyphosis 3 = Malformation of the thoracic vertebral body 4 = Umbilical hernia 5 = Clubfoot on the left aplasia of the metatarsals and phalanges III and IV.

Tabelle 5. *Embryotoxische Wirkung persischen Traganths für NMRI-Mäuse I = 0,2ml/Tier i.p. als 1%iger wäßriger Schleim (Tötung der Muttertiere am 19. Trachtigkeitstag)*

Traganth Nr.	a Applikationstag p.c.	b Muttertierzahl ges.	b Muttertierzahl mit mißgeb. Feten	e Implantationen ges.	e Implantationen 0	g Resorptionen ges.	g Resorptionen %	Aborte ges.	Aborte %	j Feten k lebend ges.	k lebend 0	l Gewicht g 0	m Länge cm 0	n tot ges.	o mißgebildet ges.	o mißgebildet %	p Art der Mißbildung
2	11	8	3	85	10,6	11	12,9	0	0	72	9,0	1,1	2,1	2	6	7,1	6 · 1
6	11	10	0	104	10,4	58	55,8	13	12,5	27	2,7	1,0	2,1	6	0	0	
7	11	6	0	64	10,7	46	71,8	9	14,1	9	1,5	1,2	2,3	0	0	0	
0	12	15	5	150	10,0	45	30,0	0	0	105	7,0	1,2	2,2	0	10	6,7	9 · 1 1 ·
2	12	11	5	116	10,5	17	14,7	5	4,3	93	8,5	1,1	2,2	1	14	12,1	13 · 1 1 ·
6	12	8	2	81	10,1	55	67,9	0	0	22	2,8	1,0	2,0	4	3	3,7	3 · 1
7	12	8	0	84	10,5	22	26,2	62	73,8	0	0	0	0	0	0	0	
0	14	5	0	45	9,0	41	91,1	4	8,9	0	0	0	0	0	0	0	
2	14	3	2	30	10,0	5	16,7	0	0	24	8,0	1,1	2,2	1	3	10,0	3 · 1
6	14	8	2	90	11,3	20	22,2	0	0	22	2,8	1,0	2,1	48	7	7,8	5 · 1 1 · 1 · 2
7	14	5	0	52	10,4	0	0	31	59,6	4	0,8	1,0	2,1	17	0	0	

ges. = Gesamtzahl pro Gruppe, 0 = Durchschnitt pro Muttertier, % = bezogen auf Gesamtimplantationszahl, 1 = Gaumenspalte 2 = Kyphose, 3 = Brustwirbelkörpermißbildung, 4 = Umbilicalhernie, 5 = Klumpfuß links Aplasie der Metatarsalia und Phalange III und IV.

Abb. 2. Links: Fet mit hypoplastischer Fehlbildung der hinteren Extremität und Tibiaaplasie. Behandlung der Muttermaus: einmal 0,2 ml pro Tier i.p. eines 1%igen wäßrigen Schleims aus persischem Traganth am 12. Trächtigkeitstag. Rechts: Normaler Kontrollfet. Muttermäuse wurden am 19. Trächtigkeitstag getötet. Aufhellung und Anfärbung des Skeletsystems nach einer modifizierten Dawson-Methode

In these tests, the strongest ~~embryo~~feto toxic effect was exerted by Charge No. 7, but even after injection of the tragacanth-Charge No 6 on the 11th or 12th day of gestation, the majority of the fetuses were resorbed or expelled as miscarriages.

After the injection of this tragacanth (No. 6) on the 14th day of gestation, 20 fetal resorptions, 22 alive and 48 dead fetuses were found in the uteri of the 8 mother mice treated. One of the 22 living, and 4 of the 48 dead fetuses had cleft palates. Furthermore, kyphosis was detected in a dead fetus, and in another a malformation of the thoracic vertebral body. The seven malformed living and dead fetuses originated from two of the eight mother mice.

The Persian tragacanth specimens No. 0 and 2, used for these tests, produced fetal resorption and abortions with lower frequency; nevertheless, 29 of the 294 living fetuses and 2 of the dead ones had cleft palates. The 10% proportion of cleft palates is 10 times higher than the spontaneous cleft palate frequency (1.02%) of our NMRI mice-breed. The 29 living and 2 dead fetuses with cleft palates come from 14 different mother animals.

Cleft palates occurred most frequently in the mother mice treated on the 12th day of pregnancy with Persian tragacanth mucilage No. 0 and No. 2; then in 10 of the 26 mother animals, fetuss(21 living and 1 dead) were found with a fissure of the palate. Furthermore, one fetus of these 10 mother animals had an ectopia of the abdominal organs. In another fetus with uranoschism, once the skeleton had been colored, a hypoplastic malformation of the right rear extremity with tibial aplasia was detected (Figure 2). Thus altogether in 10 of the 26 mother mice treated on the 12th day of gestation, 23 living fetuses; and one dead, were found with malformations.

Figure 2: Left: Fetus with hypoplastic malformation of the read extremity and tibial aplasia. Treatment of the mother mouse: one 0.2 ml per animal intraperitoneally of a 1% aqueous mucilage from Persian tragacanth on the 12th day of gestation. Right: Normal control fetus. The mother mice were caused to die on the 19th day of pregnancy. Clarification and coloration of the skeletal system by a modified Dawson method.

A 1% aqueous mucilage of Persian tragacanth of different origin(s) exerted an embryotoxic effect not only after the repeated, but already after the single intraperitoneal injection of 0.2 ml per animal in the middle of the gestation in the case of the NMRI mice used for the investigations.

In order to determine whether tragacanth from other regions of cultivation possesses the same embryotoxic effect as Persion tragacanth, two different specimen of Indian tragacanth (No. 1 and 2) were tested in the same way as in Experiment 1.

Unlike Persian tragacanth, these Sterculia-rubber specimens, even after the five-time injection of 0.2 ml per animal from the 11th to the 15th day of gestation - a dose which in the case of the Persian tragacanth always caused 100% fetal death - failed to affect the fetal development of the mice. Only the proportion of resorptions was slightly raised as compared with the normal occurrence, and 7 of the 239 fetuses (2.9%), from 7 different mother animals, had uranochism.

The repeated five time subcutaneous injection of each time 0.2 ml per animal from the 11th to the 15th day of gestation of a 1% aqueous mucilage, obtained from Persian tragacanth No. 2, failed to affect the fetal development of all the 12 mother mice.

Hence, it must be assume that the embryotoxic effect (observed after intraperitoneal injection) of different tragacanth charges of Persian origin is conditioned not by a systemic, but by a direct action on the uterus.

Since some of the investigated suspensions are used also in manufacturing suspensions to be orally applied, and since tragacanth as well as guar and agar are allowed as food thickeners, the fetotoxic effect of these natural substances, as compared with gum arabic and talc, was tested by oral application also on 35 mice. The soundings (probings) were undertaken from the 11th to the 15th day of gestation daily in amounts of 0.5 ml per animal in the form of 1% and 10% suspensions or mucilages. Despite this high dosage, the proportion of fetal resorption, the average number of living fetuses,

their body lengths and weights as well as the malformation rate in the tests with Persian and Indian tragacanth, agar and gum arabic, lay within normal limits. It is only after the sounding of 10% talc suspension and of 1% guar mucilage that the proportion of fetal resorption was raised.

Thus Persian tragacanth in oral application of large amounts even, like the other natural substances tested, failed to affect the fetal development of the NMRI mice.

3. Embryotoxic Effect of Suspensions in Early Pregnancy:

Previous tests with chemical, teratogenic compounds like formamide, monomethyl formamide, 1-ethylene imino-2-oxybutene-3 and 6-mercaptopurine showed that during the early period of gestation the mice-embryos were only slightly sensitive to teratogenic noxas (OETTEL and FROHBERG, 1964/1965). Hence tests were conducted in order to determine whether the embryotoxic effect of Persian tragacanth after intraperitoneal injection during the first third of the gestation is weaker than after application between the 11th and 15th day of gravidity.

Sixteen mice were injected from the 4th to the eighth day of gestation with 0.2 ml of a 1% aqueous mucilage from Persian tragacanth intraperitoneally. For comparison purpose, mice were treated in the same way with distilled water or mucilages from different natural substances (agar, guar, gum arabic, Indian tragacanth). Like in the previous tests, the animals were caused to die on the 19th day of gestation, that is one day before the normal littering term.

In all the 31 mice treated with distilled water, agar, gum arabic or Indian tragacanth - apart from the 17.6% amount of resorption some what increased in the "agar amimals" as compared with what is normal - the fetal development was not disturbed. The number of fetuses, their weight and length, the proportion of malformations and resorptions corresponded to those of the untreated mice. On the other hand, normally developed fetuses were found only in one of the 16 animals treated with Persian tragacanth. The uteri of the other 15 mother mice, when these were killed on the 19th day of gestation did not differ from those of nonpregnant animals.

The same dose, administered on the 6th and 7th day of gestation, caused vaginal bleedings in 3 of the 7 mother animals. Hence, these mice, and also those treated on the 7th and 8th day of gravidity, were caused to die not one day before littering, but already on the 12th day post coitum. At this moment, fetuses (66) still living in the uteri were found only in 6 of the 13 mice. In 4 of the 13 mothers, all the fetuses had been expelled. It was possible, however, to determine the number of the expelled embryos by observing the places of implantation that were still visible - in the form of "metrial glands".

On the other hand, in 6 out of 16 mice, which had been treated from the 4th to the 8th or from the 4th to the 7th day of gestation with tragacanth mucilage and had been killed immediately, the uteri did not differ from those of nonpregnant mice. Since, however, in 2 of the 16 animals, normal embryos, corresponding in their development to the 7th or 8th day of gestation, were present in the uteri, and since in the other mice the places of implantation were clearly recognizable on the mesometrium insertion, it must be assumed that in the 6 animals without "metrial glands" the embryos were expelled already before, or at the beginning of, the embedding process.

Thus the embryotoxic effect of Persian tragacanth was weaker in the case of injections administered during early gestation than in application in the middle of the gravidity period. As is shown by the tests, the mice-embryos with increasing age become more sensitive to the embryotoxic effect of Persian tragacanth; whereas Persian tragacanth mucilage injected on the 4th and 5th day of gestation failed to disturb the embryo development, fetal death and miscarriage or resorption occurred after injection on the 6th and 7th day of gestation, and still more markedly after application on the 7th and 8th day of gravidity.

4. Comments on the Problem of the Antineoplastic Effect of Suspensions:

ROE (1959), GALBRAITH, MAYHEW and ROE (1962) as well as MAYHEW and ROE (1964) found that native Persian tragacanth, but not heated Persian tragacanth and Karaya rubber (= Indian tragacanth) inhibited the growth of mice-ascites-tumors.

On the basis of mitosis counts on the Landschütz-ascites-tumor of the mice, MAYHEW and ROE (1964) surmized that the cause of the antineoplastic effect (observed by them) of the native true tragacanth was a mitosis inhibition and an "active component" of this drug, influencing the surface of the tumor cells. Hence tests were conducted in order to determine whether the embryotoxic effect, observed in the case of the Persian tragacanth, was an expression of the cytotoxic action described by ROE and Collaborators for tragacanth. For this purpose, Persian tragacanth (No. 0) was subjected to experimentation regarding antineoplastic action on the Ehrlich-ascites-carcinoma of the mouse, as compared with other suspensions and natural substances.

For each series of tests, 40 to 60 mice were used. All the animals were inoculated intraperitoneally with fresh ascites 1:1 or 1:10 diluted with Ringer's solution. Each test or control group comprised 10 mice; the controls remained untreated. All experimental animals - starting two hours after the tumor transplantation - were inoculated intraperitoneally on 5 consecutive days with 0.2 ml per animal of distilled water or of a 1% aqueous suspension of the corresponding suspensions. The mice were weighed daily, they were observed until their spontaneous death; then they were dissected. The ascites was removed by sponging the abdominal cavities with cellulose, and its amount was determined by subsequently weighing the animals. The evaluation of the antineoplastic effect was made on the basis of the ascites amount found, and also of the surviving period of the tumor animals (OETTEL and WILHELM, 1957). See individual results on Table 7.

Table 7: Antineoplastic effect of natural substances on the Ehrlich-ascites-carcinoma of the mouse. Administration of 5 x 0.2 ml/animal intraperitoneally of a 1% aqueous suspension (Monday until Friday). (First injection two hours after the tumor transplantation).

a. Substance b. Life duration in days c. Average value d. Absolute range of dispersion e. Amount of ascites at death f. Tumor inhibition in % g. Test series I h. Untreated i. Distilled water j. Gum arabic 10% k. Talc 10% l. Indian tragacanth m. Persian tragacanth o. agar (threads) p. Carrageen q. Guar

In the tumor transplantation, 1 ml of diluted fresh ascites was injected intra peritoneally into the mice. Ringer's solution was used for dilution. Dilution ration 1:1 in the series of tests I, and 1:10 in II and III.

Tabelle 7. *Antineoplastische Wirkung von Naturstoffen am Ehrlich-Ascites-Carcinom der Maus. 5 × 0,2 ml/Tier i.p. einer 1%igen wäßrigen Suspension (Montag bis Freitag). (Erste Injektion 2 Std nach der Tumortransplantation)*

a Substanz	b Lebensdauer in Tagen		e Ascites-menge beim Tod (g)	f Tumor-hemmung in %
	c Mittel-wert	d Absolute Streuung „range"		
g *Versuchsreihe I*				
h Unbehandelt	11,9	8—15	9,6	—
i Aqua dest.	11,5	7—15	9,1	4.4
j Gummi arabicum 10%	8.2	6—12	4,1	57,6
k Talkum 10%	9,1	7—12	5,2	45,3
l Ind. Traganth (Nr. 2) 1%	9,0	8—15	5,0	47,5
m Pers. Traganth (Nr. 0) 1%	6,8	4— 8	1,6	83,2
Versuchsreihe II				
Unbehandelt	14,9	12—18	9,3	—
Aqua dest.	12,8	7—17	8,0	13,7
Gummi arabicum 10%	11,3	9—14	5,1	45,1
Talkum 10%	10,7	4—15	4,4	52,5
Ind. Traganth (Nr. 1) 1%	10,2	8—13	4,2	54,4
Pers. Traganth (Nr. 0) 1%	8,1	5—14	0,5	94,7
Versuchsreihe III				
Unbehandelt	11,6	9—15	6,2	10,5
q Agar (Fäden) 1%	9,1	8—13	2,0	67,7
p Carrageen 1%	11,0	8—16	5,6	10,5
r Guar 1%	9,1	8—12	2,8	55,2

Bei der Tumortransplantation wurde den Mäusen jeweils 1 ml verdünnter, frischer Ascites i.p. injiziert. Zur Verdünnung wurde Ringerlösung verwandt. Das Verdünnungsverhältnis betrug in der Versuchsreihe I 1:1, in den Versuchsreihen II und III 1:10.

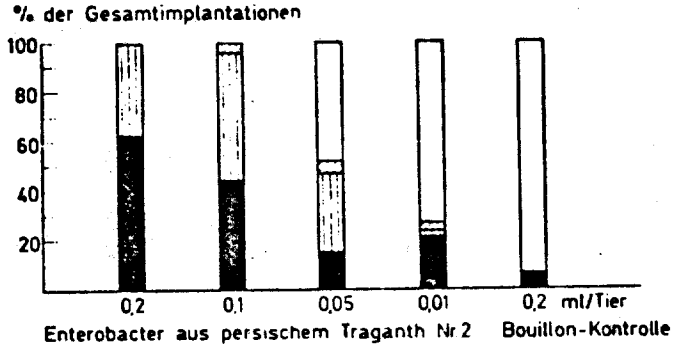

Abb. 5. Embryotoxische Wirkung kleinerer Dosen von Enterobacter-Bouillon-Sterilfiltraten für NMRI-Mäuse einmal i.p. am 12. Tag p.c. ■ Resorptionen, □ Aborte, ▭ Mißbildungen, ▭ lebende Feten

With the exception of the carrageen animals, in all the series of tests, in the mice treated with the different suspensions, the ascites amount decreased by 50% as compared with the controls, and even by 80 to 90% in the animals treated with Persian tragacanth. This decrease of the ascites amount, however, implied in every case the shortening of the surviving period of the experimental animals as compared with the controls, and thus does not express a true tumor inhibition.

5. Effects of Heat Sterilization and of Antibiotics on the Embryotoxic Action of Persian Tragacanth:

In trying to find the causes of the embryotoxic effect of Persian tragacanth, tests were performed in order to ascertain whether this effect was due possibly to microbial contaminations. For this purpose, a specimen of Persian tragacanth (No. 2) and for comparison a specimen of Indian tragacanth (No. 1) were dry-sterilized 30 minutes at 170 C. Then, from these two tragacanth specimens and with distilled water in each case a 1% mucilage was manufactured which was used in treating intraperitoneally mice from the 11th to the 15th day of gestation. Furthermore, mother mice were treated in the same way with an aqueous mucilage from Persian tragacanth which had been previously incubated for 48 hours at 37 C after adding penicillin (100 I.U./ml) and streptomycin (100 µg/ml) (See Figure 3).

Figure 3: Embryotoxic effect of pretreated Persian tragacanth for NMRI mice 5 x 0.2 ml/animal intraperitoneally as 1% aqueous mucilage from the 11th to the 15th day p.c.(?) Resorptions Miscarrages Dead fetuses Living fetuses

a. % of the total implantations b. Untreated c. Antibiotics d. Sterilized

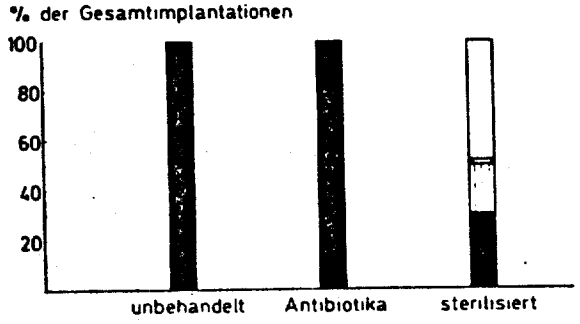

Abb. 3. Embryotoxische Wirkung vorbehandelten persischen Traganths für NMRI-Mäuse 5 · 0,2 ml/Tier i.p. als 1%iger wässriger Schleim vom 11. - 15. Tag p.c. ■ Resorptionen, □ Aborte, □ tote Feten, □ lebende Feten

Through a 30 minute sterilization at 170 C, the embryotoxic effect of Persian tragacanth was decreased, since even after the five time injection of this mucilage, manufactured from sterilized true tragacanth, only half the fetuses were resorbed or were expelled as abortions. As was expected, heat-sterilized Indian tragacanth influenced the fetal development just as little as untreated (Indian tragacanth).

A penicillin and streptomycin addition failed to lower the embryotoxic effect of Persian tragacanth.

6. Bacteriological Investigation of the Different Tragacanth Specimens:

Since it was possible to lower the embryotoxic action of Persian tragacanth through heat sterilization, it was presumable that microorganisms or their products of metabolism might be the cause of the embryotoxic effect of Persian tragacanth. Hence, the different charges of Persian and Indian tragacanth were examined bacteriologically.

In order to isolate the microorganism contained in the individual tragacanth charges, 1% mucilage specimens were prepared under sterile conditions with sterile physiological salt solution. From these specimens, after a 48 hour incubation at 37 C, a loop was inoculated on nutrient agar and the dishes were incubated at 37 C for 24 to 48 hours. Furthermore from the different 1% mucilage specimens, 1 ml in each case was placed in 50 ml of nutrient broth, and thus was processed in the same way.

In order to determine the presence of possible impurites through fungi, the same tragacanth specimens were inoculated also on Sabouraud nutrient media and Sabouraud broth, and were incubated for 14 days at 22 C.

From the germs, grown on the agar dishes and in the broth cultures, pure cultures were obtained, and the different types of germs were examined morphologically, biochemically, and in the case of the gram negative bacteria serologically also (specimen agglutination with salmonella cumulative serum I-Behring Plant).

In order to obtain an accurate determination of the isolated gram negative germs, the following properties were tested regularly, or the following reactions were conducted:

Motility
Hemolysis
Gelatin liquefaction
H_2S formation
Urea dissociation (splitting)
Methyl red reaction
Voges-Proskauer reaction
Indole reaction
Koser citrate
"Multicolored Series" with the following hydrocarbons and alcohols: dextrose, lactose, saccharose, salicin, inositol, adonitol, dulcitol.

For further differenciation, in individual cases, the following culture characteristics were investigated also:

Splitting of sorbitol, xylose, arabinose, starch.
Behavior in litmus milk, nitrate reduction, detection of
Phenylalanine-decarboxylase, lysine-decarboxylase, catalase, cytochrome-oxydase.

In the case of the gram positive bacilli, the following culture characteristics were used for diagnostic purpose:

Growth in broth and on agar
Motility
Hemolysis
Gelatin liquefaction
Splitting of dextrose, lactose, saccharose, xylose, mannitol
Indole reaction
Voges-Proskauer reaction.

In the case of the isolated gram positive diplococci, the following investigations were conducted:

Growth in broth and on agar
Hemolysis
Splitting of dextrose, saccharose, lactose, mannitol, maltose, salicin, sorbitol, induline, aesculine
Indole reaction.

On the basis of the undertaken investigations, the germs, isolated out of the different tragacanth specimens, were determined as follows: Results in Table 8.

Table 8: Bacteriological findings relative to different tragacanth specimens
a. Tragacanth specimen b. Yeasts c. Fungi

a. Traganth-probe	b. Bakterien		c. Hefen Pilze
	gramnegativ	grampositiv	
Pers. Traganth			
1	Enterobacter aerogenes	—	—
2	Enterobacter aerogenes	—	—
3	Enterobacter aerogenes	—	Penicillium
4	Enterobacter aerogenes	—	—
5	Enterobacter aerogenes	—	—
6	Enterobacter aerogenes Flavobact. rhenanus	Bac. circulans Bac. subtilis Diplococcus (nicht differenziert)	Mucor
7	a) Enterobacter aerogenes b) Enterobacter aerogenes	Bac. megatherium Diplococcus (nicht differenziert)	—
Ind. Traganth			
1	—	Bac. mesentericus Bac. subtilis	— —
2	—	Bac. mesentericus Bac. subtilis	—

After these investigations, enterobacter aerogenes germs were detectable in all the Persian tragacanths, and even as pure culture in specimens 1 to 5.
In the gragacanth specimen No. 6, a flavobacterium rhenanus, belonging to the family of the achromobacteraceae, was still isolated. In the tragacanth specimens

No. 6 and 7, it was possible to detect, in addition to enterobacter-aerogenes germs and the flavobacterium, gram positive aerobic sporiferous (bacteria ?) as well as gram positive diplococci, which were not more closely differentiated. In the tragacanth specimens No. 3 and 6, a penicillium or a Mucor germ was detectable; the latter was not included in the further investigations.

In contrast with the results of bacteriological investigations, in the Persian tragacanth specimens of different origins, no germs had grown in the parallel text with the two Indian tragacanths after an incubation of 24 hours. It was only after a storage of 36 to 48 hours at + 37 C, that on the nutrient agar dishes different gram positive aerobic sporiferous bacteria (Bac. subtilis, Bac. mesentericus were detected. The only low number of bacteria in the Sterculia rubber should be attributed to the fact that the commercial product is solubilized through the autoclave (Merck Index, 8th Edition 1968).

According to the investigations conducted so far, the gram negative germs contained in the Persian tragacanth specimens might be regarded as the cause of the embryotoxic effect. Hence, tests were performed in order to determine the possibility of isolating these gram negative bacteria from the absominal cavity, after repeated intraperitoneal tragacangh injection.

For this purpose, two mice, which had been treated intraperitoneally one, 3 or 5 times with 0.2 ml of a 1% mucilage of Persian or Indian tragacanth, were caused to die nearly 8 hours after the last injection; the abdominal cavity was opened under sterile conditions, the peritoneal fluid between the partly adherent intestinal loops were removed, and inoculated on agar dishes.

It was possible to isolate the enterobacter germs, detected bacteriologically in the different Persian tragacanth specimens - although these germs were few - from the absominal cavity of the mice treated intraperitoneally 4 or 5 times with these mucilage specimens. In the test with Persian tragacanth No. 6, the gram positive diplococci were found again in the abdominal cavity of the animals treated with this tragacanth. Nevertheless, it was impossible to isolate the gram positive aerobic sporiferous bacteria, primarily detected in the Persian tragacanth specimens No. 6 and 7 or in the Indian tragacangh No. 1, from the

abdominal cavity of the mice treated repeatedly intraperitoneally in the case of these specimens.

7. Embryotoxic Effect of Products of Metabolism of Bacteria from Persian Tragacanth:

In order to exclude a bacterially induced peritonitis as cause of the embryotoxic effect of Persian tragacanth, tests were conducted in order to ascertain whether products of metabolism of the isolated germs can also exert an embryotoxic effect. For this purpose, normal nutrient broth was inoculated with pure cultures of the gram negative germs isolated from the different Persian tragacanth specimens (2 Pt loops of a 24-hour culture on 50 ml of broth) and was incubated during 48 hours at +37 C. Then these broth cultures were passed under sterile conditions through bacteria-proof filters (Seitz/Filter - Layer EKS II (EKS = sterilization layer)). After verifying the sterility by preparing smears on agar dishes, these broth cultures made sterile by filtration were injected under sterile conditions with the products of metabolism of the enterobacter strains in mice intraperitoneally - starting on the 11th day of gestation - in doses of 0.2 ml per animal (Figure 4).

Figure 4: Embryotoxic effect of broth cultures, filtrated under sterile conditions, of enterobacter strains for NMRI mice. 1 x 0.2 ml/animal intraperitoneally on the 11th day of gestation Resorptions, Miscarriages, Dead fetuses, Living fetuses, Malformations.

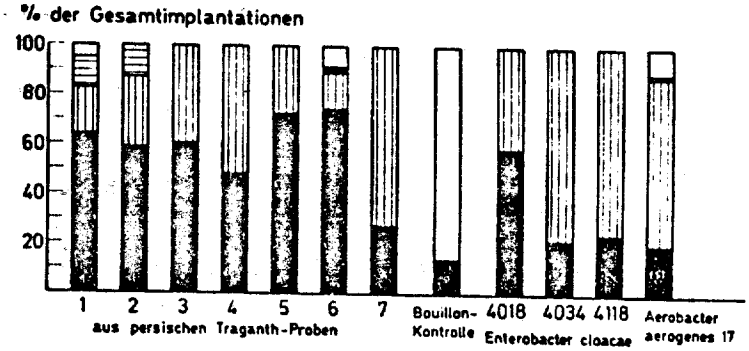

Abb. 4. Embryotoxische Wirkung steril filtrierter Bouillon-Kulturen von Enterobacter-Stämmen für NMRI-Mäuse. 1 × 0,2 ml/Tier i.p. am 11. Tag p.c. Resorptionen, Aborte, tote Feten, lebende Feten, Mißbildungen

Already in the course of 12 to 15 hours after the first intraperitoneal injection, vaginal bleedings - which must be regarded as sign of miscarriage - developed in 7 of the experimental groups, which had been treated with enterobacter broth culture filtrates from the Persian tragacanth specimens No. 1 to 7, in the majority of the mice. Hence, the mice of these 7 experimental groups, which for a certain time (1 - 2 days) exhibited a ruffled skin and retracted flanks, were treated only once. Three mother animals died intercurrently.

Despite the only single intraperitoneal injection of the Persian tragacanth specimens No. 1 - 7, living fetuses were found in uteri only in 2 of the 55 mother mice treated. All the other 543 fetuses had been resorbed or had been expelled as abortions.

The 13 mice of the two other experimental groups developed no vaginal bleedings, hence they were treated from the 11th to the 15th day of gestation. In this experimental setup, the two sterile filtrates, which contained products of metabolism of the flavobacterium rhenanus and of an enterobacter aerogenes, exerted an embryotoxic effect, since about half the fetuses of the mother animals were rsorbed or expelled as miscarriage. The embryotoxic action of the products of metabolism of this flavobacterium rhenanus and of this enterobacter strain, however, was considerably weaker than that of the other enterobacter strains, which already after the single injection practically caused the death of all the fetuses. In its cultural properties also, the enterobacter germ differed from those of the other enterobacter strains.

These investigations reveal that the sterile filtrates of broth cultures, inoculated with enterobacter germs and incubated for 48 hours at +37 C, exerted an embryotoxic effect.

In order to determine whether other strains of the same genus had an embryotoxic effect, sterile filtrate of broth cultures of the aerobacter aerogenes strain 17 (Dr. F. Selenka) and of 3 enterobacter cloacae strains (Dr. B. Schmidt) were tested (The long footnote at this point merely expresses thanks to these two university lecturers).

The sterile filtrates of the enterobacter strains defined caused the death of 241 out of 253 fetuses of the 34 mother mice, after a single injection on the 11th day of gestation (See Figure 5 P. 14a).

A 1% aqueous mucilage from Persian tragacanth, after repeated subcutaneous injection, had no embryotoxic effect. Hence, a broth culture made sterile by filtration was prepared from the same gragacanth charge in the way described above. This filtrate was injected under sterile conditions subcutaneously in 18 mice from the 11th to the 15th day of gestation in doses of 0.2 ml/animal. Furthermore 5 gravid mice were treated in the corresponding way with a broth culture made sterile by filtration; this filtrate had been prepared with enterobacter cloacae 4034. The 8 control mice received individually 0.2 ml of empty broth.

The fetal development of the 23 mother animals, treated with sterile filtrates of broth culture (obtained from Persian tragacanth No. 2 and from enterobacter cloacae 4034), did not differ from the 8 control mice treated with empty broth.

These "subcutaneous tests" corroborated the assumption that the embryotoxic effect (observed after intraperitoneal injection in NMRI mice) of tragacanth contaminated with bacteria of the genus enterobacter, is due not to a systemic but to a direct action on the uterus and on its vacular system.

In the tests reported at the beginning of this study, a single intraperitoneal injection of a 1% Persian tragacanth mucilage was followed, especially on the 12th day of gestation by increased malformations and especially by cleft palates (See Table 6). Hence, tests were conducted in order to determine whether the products of metabolism of enterobacter in doses, which practically no longer killed all the fetuses or produced resorption or miscarriage, possibly caused increased malformations.

For this test, a sterile filtrate, which had been obtained from a broth incubated for more than 48 hours with enterobacter strain 2, was injected in gravid mice in doses of 0.2; 0.1; 0.05 and 0.01 ml per animal once on the 12th day of gestation.

Like in the previous tests, on the 11th day of gestation, after a single intraperitoneal injection on the 12th day of gestation of 0.2 ml/animal of the sterile filtrate of broth culture, practically all the fetuses were resorbed or were expelled as miscarriage. The two surviving fetuses of the "0.1 ml group" had uranoschism. After the injection of 0.05 ml/animal, about half of the fetuses were killed, and even after the administration of only 0.01 ml/mouse still 16 of the 75 fetuses were resorbed, and 2 were expelled as miscarriage. Four of the 79 living fetuses of these two series of tests had malformations, especially cleft palates and some malformations of the extremities with extended subcutaneous bleedings.

In order to exclude the possibility for products of metabolism of the gram positive germ found in the different tragath specimens to possess an embryotoxic action, broth cultures made sterile by filtration were prepared under the same conditions as in the case of the gram negative germs from the different gram positive aerobic sporiferous bacteria (Bac. mesentericus and subtilis) and diplococci, which had been isolated from Persian and Indian tragacanth; and these cultures were used in injecting intraperitoneally mice from the 11th to the 15th day of gestation with 0.2 ml/animal daily.

In the same way, sterile filtrates of broth cultures of different other germs, stemming from the collection of the Industrial Hygiene Pharmacological Institute, such as E. coli I, Staph. aureus SG 511, Bac. subtilis and Bac. mesentericus, were tested.

Neither the sterile filtrates of broth cultures of the gram positive germs isolated from Persian and Indian tragacanth, nor the concomitantly tested control filtrates of E. coli, Staph. aureaus SG 511, Bac. subtilis and Bac. mesentericus possessed an embryotoxic action on the 103 gravid NMRI mice used for these tests.

8. Effect of the Use of the Autoclave on the Embryotoxic Action of Sterile Filtrates of Enterobacter Broth Cultures:

In order to find out whether through the use of the autoclave the embryotoxic action of products of metabolism contained in the sterile filtrates of broth cultures of enterobacter strains is decreased or eliminated, sterile filtrates of broth cultures of the strains Aerobacter aerogenes strain 17, Enterobacter cloacae 4034, Enterobacter strain from Persian tragacanth No. 2 and Enterobacter strain from Persian gragacanth No. 6 were subjected to a single treatment for 15 minutes at 120 C in the autoclave. These sterile filtrates of broth culture, thus treated, were injected once daily intraperitoneally in gravid mice once 0.2 ml/ animal on the 11th day of gestation or from the 11th to the 15th day of gestation in amounts of 0.2 ml/ animal (See Figure 6).

Figure 6: Embryotoxic effect of broth cultures (obtained by sterile filtration) from Enterobacter strains after the use of the autoclave for NMRI mice.

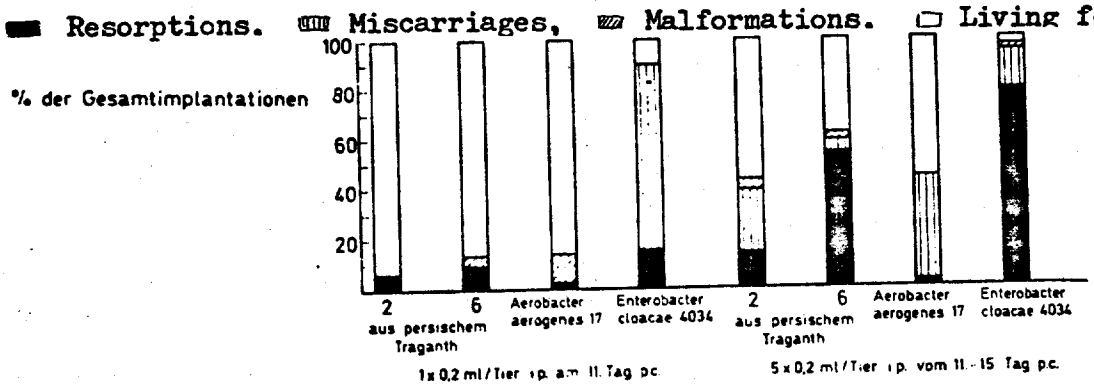

Abb. 6. Embryotoxische Wirkung steril-filtrierter Bouillon-Kulturen von Enterobacter-Stämmen nach Autoklavierung für NMRI-Mäuse. ■ Resorptionen, ▤ Aborte, ▨ Mißbildungen, □ lebende Feten

The use of the autoclave for 15 minutes weakened the embryotoxic effect of the products of metabolism of the different enterobacter strains, but failed to suppress it; after the five time intraperitoneal injection, about the half of the fetuses of the mother animals treated were resorbed or were expelled as miscarriages. The autoclaved sterile filtrate of the broth culture from Enterobacter cloacae 4034 was just as embryotoxic as the corresponding nonautoclaved sterile filtrate, since after the single injection on the 11th gestation day 77 out of 104 fetuses were expelled as abortion and 16 were resorbed.

Discussion:

Distilled water, Ringer's solution, the 1% mucilages of gum arabic, carrageen or guar, used in manufacturing suspensions or emulsions, and even 1% aqueous talc suspensions do not influence the fetal development of NMRI mice after a single intraperitoneal injection, or after several, in the sensitive phase of gestation. Under the same experimental conditions, however, once mother mices have been treated with 1% aqueous mucilages from 8 different Persian tragacanth specimens, the death and subsequent resorption or expulsion of all the fetuses occur. On the other hand, mucilage from 2 Indian tragacanth specimens exerts no effect, like the other natural substances tested, on the fetal development.

The mice-fetuses most easily injured are those involved in the treatment administered in the middle of the gestation period (11th and 12th day of gravidity). In the stage of advanced organogenesis, the fetuses become increasingly more resistant to the embryotoxic effect of Persian tragacanth. Thus the sensitivity of mice-embryos to pure tragacanth, during their development, is just as different as to known chemical or physical teratogenic noxa (WILSON, 1965; RUGH and GRUPP, 1959 as well as OETTEL and FROHBERG, 1965, 1965).

In all the mother mice, treated with a 1% aqueous Persian tragacanth mucilage intraperitoneally, dissection revealed inflammatory changes in the abdominal cavity. Hence at first it was assumed that the peritonitis, produced by the intraperitoneal injection, was the cause of the fetal injury, especially ... other natural substances, which do not affect the fetal development, like the investigated Indian tragacanth-, agar, or carrageen specimens which even after repeated intraperitoneal injection in the form of 1% aqueous mucilages do not exert a stimulating effect. There is an objection, however, against peritonitis as cause of the embryotoxic effect of Persian tragacanth: 1% aqueous gum arabic and especially talc suspensions after repeated intraperitoneal injection in mice cause stronger inflammatory stimulations in the abdominal cavity than Persian tragacanth, although these suspensions have just as little effect on the fetal development as Ringer's solution or distilled water. Even through five time intraperitoneal injection of a 10% aqueous gum arabic or talc suspension, the fetal development , despite most sovere peritonitic inflammations and massive adherences of the ingesta in the mother animals is less disturbed than through

a single injection of 1% mucilage from Persian tragacanth. Hence, the peritoneal stimulation produced by this tragacanth after intraperitoneal injection should not be the cause of its embryotoxic action.

In contrast with the intraperitoneal tests, Persian tragacanth mucilage in oral application even after repeated administration of large amounts, affects the fetal development of mice just as little as the other tested natural substances. Hence - as far as a posteriori conclusions can be drawn from animal tests and applied to human beings - there should be no hesitation, from the embryotoxic effect standpoint, to continue the utilization of Persian tragacanth, beside other natural substances, as an addition to food.

A cytotoxic effect, described by ROE (1959), GALBRAITH, MAYHEW and ROE (1962) as well as MAYHEW and ROE (1964) in the case of Persian tragacanth on the basis of investigations with some mice-ascites-tumors, must be excluded, according to the investigations presented here, as cause of the observed embryotoxic effect of pure tragacanth, since the Persian tragacangh specimens, that we tested, like the other natural substances, possess on the Ehrlich ascites carcinoma of the mouse no significant antineoplastic effect after intraperitoneal injection.

According to our investigations, it is rather bacteria or their products of metabolism that must be made responsible for the embryotoxic effect of the Persian tragacanth charges tested. This is shown already by the decrease of the embryotoxic action of aqueous mucilage from Persian tragacanth after sterilization, and especially by the presence of gram negative coccoid rods of the genus Enterobacter in all the 7 investigated Persian tragacanth specimens, which are not found in the two Indian tragacanth specimens. The considerable lack of bacteria in Sterculia-rubber should be attributed to the solubilization of this commercial product through the autoclave (Merck Index, 1968). The products of metabolism of the individual isolated Enterobacter strains, injected intraperitoneally in the form of sterile filtrates of broth cultures in gravid animals, exert a higher embryotoxic effect than the original tragacanth mucilage. The products of metabolism of bacteria of these Enterobacter strains should represent thus the real active form. The control tests, conducted with defined Enterobacter strains, show that the embryotoxic effect is specific for the bacteria products of metabolism of the isolated bacteria genus (Enterobacter).

The gram positive aerobic sporiferous bacteria and diplococci, isolated from Persian tragacanth, generate no products of metabolism with embryotoxic action. In the same way behave also the gram positive germs, which were isolated from the Indian tragacanth specimens, or had been borrowed from the collection of our Institute. The broth culture, filtrated under sterile conditions, of an E. coli-strain used for comparison did not have an embryotoxic effect either.

Hence, according to the investigations, the gramnegative germs, isolated from the different Persian tragacanth specimens, of the genus Enterobacter or their products of metabolism are the cause of the embryotoxic effect of Persian tragacant after intraperitoneal injection for NMRI mice. Nothing precise can be stated about the nature of these products of metabolism of bacteria. Since, however, the used of the autoclave for 15 minutes at 120 C only weakens, but does not eliminates, the ambryotoxic effect of sterile filtrates of broth cultures of Enterobacter, the effective substance must consist at least of a thermolabile and of a thermostable constituents. Whether the thermolabile constituents involves albumins, and the thermostable part possible polysaccharides - like this is known from endotoxins of gram negative germs, must be clarified through further investigations.

Our investigations with decreasing doses of a sterile filtrate of broth culture of enterobacter on the 12th day of gestation show that the number of the dead fetuses decreases in proportion to the dose injected; but that malformations are not increased. Hence it can be concluded that the resorptions and miscarriages, observed in our tests, very probably are no signs of a pure teratogenic action of the products of metabolism of the bacteria.

It is possible that the fetal death is due to massive placenta bleedings, caused by the products of bacteria metabolism of the Enterobacter strains. This might shown by the observation that in some mice, killed immediatly after vaginal bleeding was clinically found, fresh bleedings were detected on the surfaces of the placenta without any fetal residues (state after abortion) and in those with attached fetal sack in which the fetuses in the process of resorption lay. Placenta bleedings, connected with the death of the fetuses, were observed also by THIERSCH (1960) in pregnant rats after injection of lipopolysaccharides, obtained from gram negative bacteria. The cause of the placenta bleedings

could be a disturbance of the blood coagulation, since for dogs, intravenous injections of tragacanth mucilage are deadly on account of the effect exerted on the blood coagulation (WALTON and others, 1959). Such a mechanism could also be implied by the increase of the vascular permeability and lengthening of the time of coagulation found as a result of lipopolysaccharide action, by WESTPHAL and Collaborators (1955).

After repeated subcutaneous injection, a tragacanth mucilage, contaminated with bacteria of the genus Enterobacter, as well as the sterile filtrates of broth cultures manufactured from this tragacanth charge and Enterobacter cloacae 4034, did not influence the fetal development of the NMRI mice. Hence it must be assumed that the embryotoxic effect, observed after intraperitoneal injection in NMRI mice, of different Persian tragacanth charges, which were contaminated with bacteria of the genus enterobacter, and of sterile filtrate of broth cultures of Enterobacter, is not due to a systemic effect, as for instance a general disturbance of the blood coagulation, but to a direct action on the uterus and its vascular system.

For the practice, it results from the investigations presented in this study that:

1. Suspensions and emulsions, which can be contaminated by bacteria of the genus Enterobacter, should at much as possible not be used in testing the possible teratogenic effect of water insoluble substances on the mouse by means of intraperitoneal injection, and

2. Substances, which cause an increasing of the vascular permeability, or possess considerable vasodilatatory or vasoconstrictory effect, and must be applied parenterally, should not be injected intraperitoneally when testing the teratogenic effect, in order to prevent a fetal injury caused by a local perfusion disturbance of the uterus.

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urethan and phenobarbital. The results from the combination of different routes of administration support this last conclusion.

In the experiments in Table 2, one further aspect is seen. In one situation gum tragacanth was administered simultaneously at 24 hours with either urethan or

TABLE 1

EFFECT OF VARIOUS AGENTS GIVEN 24 HOURS EARLIER ON HEXOBARBITAL SODIUM (150 MG/KG) SLEEPING TIME[a]

Expt.	Pretreatment, route	Number of mice	Sleeping time
a	C	(9)	83.1 ± 4.4
	Gt, i.p.	(10)	* 81.5 ± 2.9
	GT, s.c.	(10)	90.1 ± 4.8
	GT, p.o.	(9)	77.7 ± 2.7
b	C	(9)	64.7 ± 4.6
	U, i.p.	(10)	* 45.6 ± 1.7
	U in GT, i.p.	(10)	68.5 ± 3.5
	U in GT, s.c.	(10)	* 51.1 ± 4.1
	U in GT, p.o.	(10)	* 36.4 ± 3.4
c	C	(7)	64.3 ± 3.5
	P, i.p.	(11)	* 38.5 ± 2.7
	P in GT, i.p.	(9)	* 48.8 ± 2.5
	P in GT, s.c.	(10)	* 49.8 ± 2.4
	P in GT, p.o.	(8)	* 38.0 ± 3.4
d	C	(9)	74.3 ± 6.7
	U, p.o.	(10)	* 41.7 ± 2.5
	U, p.o. + GT, i.p.	(9)	91.3 ± 7.3
e	C	(10)	81.1 ± 3.5
	P, p.o.	(10)	* 44.1 ± 2.4
	P, p.o. + GT, i.p.	(10)	74.6 ± 7.4
f	C	(10)	76.7 ± 6.2
	U, s.c.	(10)	* 48.0 ± 5.0
	U, s.c. + GT, i.p.	(10)	81.6 ± 5.9
g	C	(11)	91.2 ± 6.4
	P, s.c.	(11)	* 66.7 ± 5.6
	P, s.c, + GT, i.p.	(11)	100.7 ± 6.4
h	C	(10)	68.0 ± 6.7
	U, s.c.	(10)	* 44.3 ± 3.6
	U, s.c. + GT, p.o.	(10)	* 46.4 ± 3.7
	U, p.o.	(9)	* 44.4 ± 3.8
	U, p.o. + GT, s.c.	(10)	61.7 ± 3.3
i	C	(11)	98.1 ± 7.4
	P, p.o.	(10)	* 61.4 ± 6.4
	P, p.o. + GT, s.c.	(10)	* 76.6 ± 3.8

[a] C = control; GT = gum tragacanth, 0.1 ml/10 g body weight of a 1% suspension; U = urethan (1200 mg/kg); P = phenobarbital sodium (100 mg/kg); ± = standard error; * = P value less than 0.05 compared to control by t test.

phenobarbital. In this case, the effects of the urethan and the phenobarbital were blocked. On the other hand, gum tragacanth administered 24 hours after the urethan or phenobarbital (48 hour pretreatment) was certainly not as potent in blocking the effect of the urethan and phenobarbital. To reiterate, gum tragacanth given at the same time as the agents blocked the effect whereas if the agents preceded the gum traga-

canth, the latter did not block the effect. It appeared that once the system was "induced," gum tragacanth did not affect it. Fujimoto and Plaa (1961) arrived at a similar conclusion with respect to ethionine and CCl_4 action on the liver.

DISCUSSION

Quinn *et al.* (1954) have shown that the duration of the response to hexobarbital is proportional to the biologic half-life of the drug and is inversely related to the activity of the metabolizing enzyme system in the liver microsomes. Therefore, it is not unexpected to find that agents which damage the liver increase hexobarbital sleeping time and agents that increase microsomal enzyme activity for metabolizing hexobarbital shorten sleeping time. In these regards hexobarbital sleeping time affords one possible measure of liver function. On this premise, the results indicate that gum tragacanth, which had no effect on control sleeping time, has little functional effect on the liver. However, Fujimoto and Plaa (1961) have shown that ethionine and CCl_4 at low doses may not appreciably affect control sleeping time but can be shown to block the "inducing" action of urethan and phenobarbital. A similar conclusion can be made on the present gum tragacanth data. The sensitivity of the "induced" system to ethionine and CCl_4 is understandable in that some of the earliest changes to ethionine and carbon tetrachloride are notable on the microsomal fraction (Neubert and Maibauer, 1959) and the endoplasmic reticulum (Reynolds, 1963). On the other hand, why the "induced" system should be sensitive to gum tragacanth is problematical. As far as is known, gum tragacanth is rapidly taken up by the reticuloendothelial system of the liver. Yet, no direct relationship has been established between hexobarbital metabolism, "inducing" effect, and the reticuloendothelial system. Thus, in the present paper, there is no attempt to assess the similarity or difference which may exist between the ethionine and carbon tetrachloride as against gum tragacanth-produced block of the "inducing" effect.

The sensitivity of the "induced" system to blocking agents suggests that this system might serve as a test for hepatotoxic agents (Serrone, personal communication). Such a test could compare favorably in sensitivity to the sulfobromophthalein method of Kutob and Plaa (1962).

TABLE 2

EFFECT OF VARIOUS AGENTS GIVEN 24 AND 48 HOURS EARLIER ON HEXOBARBITAL SODIUM (150 MG/KG) SLEEPING TIME[a]

Pretreatment, route, time	Sleeping time
C	107.8 ± 6.6
U, p.o., 24 hr	* 60.2 ± 6.0
U, p.o., + GT, i.p., 24 hr	96.5 ± 9.0
GT, i.p., 24 hr	113.5 ± 9.7
U, p.o., 48 hr	* 53.5 ± 4.6
U, p.o., + GT, i.p., 24 hr	* 54.4 ± 4.0
C	96.4 ± 5.7
P, p.o., 24 hr	56.3 ± 3.9
P, p.o., + GT, i.p., 24 hr	* 77.2 ± 5.2
GT, i.p., 24 hr	117.2 ± 8.7
P, p.o., 48 hr	* 26.7 ± 2.1
P, p.o., + GT, i.p., 24 hr	* 41.6 ± 3.5

* Ten mice in each group. For abbreviations see Table 1, footnote *a*.

SUMMARY

Gum tragacanth has no effect on control hexobarbital sleeping time in mice. The effect of phenobarbital and urethan pretreatment to induce a shortening of hexobarbital sleeping is blocked by intraperitoneal injection of gum tragacanth. These results demonstrate the presence of a hepatic effect of gum tragacanth.

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MODE OF INHIBITORY ACTION OF TRAGACANTH POWDER ON THE GROWTH OF THE LANDSCHÜTZ ASCITES TUMOUR

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IT has been shown (Roe, 1959) that Tragacanth Powder inhibits mouse ascites tumour growth *in vivo*. Samples from various species of *Astragalus* have been tested and growth inhibitory activity is present in all. It has been shown to be greater for the higher-grade commercial powders, but does not reside in various gums of different botanical origin which have been tested, nor in a variety of other polysaccharides. The inhibitory effect is destroyed by mild chemical and physical treatment of the powder and experiments to date suggest that it depends on the maintenance of a specific structure in the main macromolecular component of the gum, i.e., in one, or a mixture of polysaccharides. Details of the above experiments will be published separately. This paper is an account of further work concerning the mode of action of Tragacanth Powder (T.P.).

T.P. inhibits mouse ascites tumour growth when injected intraperitoneally. In the experiments reported below the Landschütz ascites tumour was used, inoculated in C+ (male) or C— (male or female) mice (C.B.R.I. strain).

The present report is divided into four sections :—

I. Dead cell counts.
II. Mitotic Index counts.
III. *In vitro–in vivo* experiments.
IV. Polysaccharide staining.

I. Dead cell counts

Certain dyes are recognised as indicators of cell death. Lissamine Green has been the indicator preferred in these experiments as it is non-toxic in the concentrations employed (Goldacre and Sylvén, 1959; Holmberg, 1961).

Ascites tumour cell suspension from a single mouse was placed in both halves of a Burker counting chamber, diluted 1 : 2 in one side with an isotonic solution of the dye to act as a control, and in the other side with an isotonic solution of dye and T.P. in various concentrations. The percentages of cells stained after 5, 10, 20, 30, 40, 50 and 60 minutes could then be noted for both control and treated suspensions. Counts were made using T.P. concentrations of 0·1 and 1·0 mg. per ml. of cell suspension, dye concentrations of 1 : 200 and 1 : 2000, and tumours from 4 to 14 days old. A 4-day-old tumour is the youngest from which fluid may readily be extracted, and after about 14 days, untreated tumour-bearing mice usually die. The T.P. was added untreated, or deactivated by boiling for 5 minutes.

It was then thought desirable to repeat the dead cell counts after longer [illegible] in contact with the T.P. than the 60 minutes previously [illegible] and

without the uncertainties (e.g. due to leucocytosis) of the *in vivo* experiments. For this purpose a culture apparatus was made, consisting of two identical culture vessels provided with a gas reservoir containing 5 per cent CO_2 in air, an air pump which gently agitated the fluid in the vessels to prevent the settlement of cells on the inner surfaces, and a U.V. lamp illuminating two quartz tubes in the gas pipes so that the culture vessels remained sterile and isolated from the non-sterile pump and gas reservoir. The cultures were maintained at 36°. Samples could be removed from the vessels by inserting a long syringe needle through a rubber teat without breaking the sterile conditions. It was possible, therefore, to remove and count a series of samples at intervals during an experiment and to note the change with time in the percentage of dead cells for control and treated samples, the T.P. being in the culture medium of one vessel. Earle's medium was used (Earle, 1943), i.e. a solution of inorganic salts and glucose only, as it was not necessary to maintain the culture for long periods or for the cells to multiply, and it was considered advisable to avoid the complications introduced by animal extracts. By these methods the cultures were easily maintained in a healthy state for over 24 hours, and occasionally up to 72 hours. Cell concentrations were $1{\cdot}8$–$6{\cdot}0 \times 10^6$ per c.c. in these experiments and T.P. concentrations varied from 0·3 to 1·0 mg./ml.

However, in spite of the known inhibitory activity of T.P. *in vivo*, no significant differences could be found between the control and treated cells in any of the above experiments except possibly at 24 hours on occasions when the culture was moribund (Table I). Similar results were obtained in the few experiments in which Methylene Blue was used as an indicator of cell death.

Thus, since T.P. appears to have no toxic effect on the interphase tumour cells, it was decided to investigate possible effects on the Mitotic Index.

TABLE I.—*Percentage Deaths of Ascites Tumour Cells After Treatment* In Vitro *with T.P.* (*Indicator*, 1 : 2000 *Lissamine Green*)

	Time in minutes						
	5	10	20	30	40	50	60
Controls	1·1	1·8	2·2	2·4	2·5	2·7	2·9
0·1 mg./ml. T.P.	1·1	0·8	1·7	2·0	2·1	2·3	2·8
1·0 mg./ml. T.P.	1·7	2·0	2·3	2·4	2·5	2·7	2·8
1·0 mg./ml. T.P., de-activated	1·0	1·7	2·1	2·2	2·2	2·4	2·7

	Time in hours						
	0	1	2	3	4	6	24
Controls	1·4	1·4	0·9	3·3	2·4	3·3	17·6
0·3 mg./ml. T.P.	1·6	1·0	0·8	1·7	2·7	—	—
0·5 mg./ml. T.P.	0·9	0·5	—	2·9	—	4·3	24·9
1·0 mg./ml. T.P.	1·3	0·8	0·6	2·0	1·8	—	22·9

II Mitotic Index counts

For this purpose aceto-orcein squashes were made at daily intervals from the ascites tumours of control mice and mice treated intraperitoneally with different doses of T.P. at the 7th day after tumour inoculation. Two factors confused the results: first, the great increase in neutrophils and lymphocytes in the treated mice and, to a lesser extent, in the controls as the tumour aged; and secondly, the presence in the treated samples of ascites cells with pycnotic nuclei of two

varieties, dense and lobed (d and l respectively in Fig. 1) which can be confused with the lymphocytes and neutrophils respectively. The appearance of the cytoplasm can be used to distinguish these, however, for the ascites cells are larger and have more granular cytoplasm than the while blood cells. Pycnosis is partly a matter of definition, since abnormal cells can be recognised which may not be considered fully pycnotic. A provisional definition was made whereby the absence of a recognisable nucleolus denoted a pycnotic nucleus.

The results are shown in Tables II (a) and (b) and Table III. From Table II (a) it can be seen that T.P. in the higher doses completely inhibits mitosis, while in lower doses there is moderate inhibition with recovery by the third day. Boiling the T.P. destroys the mitotic inhibitory activity. In one experiment, the stages of mitosis were also counted (Table II (b)). It can be seen that all stages, even prophase, are much less frequent in the treated cells. From Table III it is clear that the percentage of pycnotic nuclei increases with increasing doses of T.P. There is a large increase in the number of neutrophils and, to a lesser extent, in the lymphocytes also.

TABLE II (a).—*Mitotic Index Changes with Time for Different Doses of Tragacanth Powder per Mouse (Administered Intraperitoneally ; 7-day-old Tumour)*

		24 hr.	48 hr.	72 hr.	96 hr.
Mitotic index per cent	Controls	1·1	1·5	1·1	1·2
	1 mg.	0·2	0·2	0·8	—
	2 mg.	0·3	0·2	1·1	1·3
	4 mg.	0·0	0·0	0·0	0·0
	4 mg. de-activated	0·8	1·2	1·5	1·1
Thousands of Ascites cells counted	Controls	34	30	18	10
	1 mg.	20	20	8	—
	2 mg.	14	10	4	4
	4 mg.	8	10	6	2
	4 mg. de-activated	8	8	8	8

TABLE II (b).—*Percentages of Ascites Tumour Cells in Various Mitotic Stages.*

	Prophase	Metaphase	Anaphase	Telophase	Cells counted
Controls, 24 hr.	0·33	0·51	0·14	0·09	6000
48 hr.	0·63	0·76	0·10	0·16	8000
Treated, 24 hr.	0·09	0·03	0·00	0·00	8000
(1 mg.) 48 hr.	0·00	0·00	0·02	0·00	8000

Fig. 1 shows the appearance of fixed and stained ascites tumour cell squashes from control and treated mice. Control cells show their normal appearance. Treated cells have numerous droplets in the cytoplasm and frequently pycnotic nuclei and in the later stages there is extensive cell breakdown.

III. In vitro–in vivo *experiments*

It was of interest to discover whether, if T.P. was applied to the cells for a short time and then removed, its anti-tumour activity would be shown, and if so, what was the minimum period of application. Therefore, a series of experiments was performed in which ascites tumour cells were maintained for varying periods in the culture apparatus either with (B, Table IV) or without T.P. (A ; i.e. controls). The cultured cells were then removed, washed with saline, spun twice in

TABLE III.—*Changes with Time in the Percentages of Pycnotic Ascites Tumour Cells, Neutrophils and Lymphocytes for Different Doses of Tragacanth Powder (Conditions as in Table II)*

		24 hr.	48 hr.	72 hr.	96 hr.
Pycnotic ascites tumour cells	Controls	1·1	1·1	1·2	0·6
	1 mg.	3·1	6·6	5·2	—
	2 mg.	3·1	3·6	5·3	10·5
	4 mg.	13·7	5·0	13·4	18·9
	4 mg. de-activated	0·3	0·4	0·3	0·4
Neutrophils, per cent	Controls	2·0	5·8	3·0	2·0
	1 mg.	8·4	15·1	7·1	—
	2 mg.	6·4	33·3	12·7	14·1
	4 mg.	20·5	64·5	53·3	46·6
	4 mg. de-activated	2·2	2·6	2·2	1·9
Lymphocytes, per cent	Controls	3·6	2·4	2·1	1·1
	1 mg.	3·8	3·1	2·7	—
	2 mg.	2·0	8·0	9·5	17·2
	4 mg.	7·9	12·2	13·7	13·2
	4 mg. de-activated	1·2	0·9	1·3	1·0
Thousands of cells counted	Controls	26	26	18	10
	1 mg.	16	16	8	—
	2 mg.	10	10	4	4
	4 mg.	8	8	6	2
	4 mg. de-activated	8	8	8	8

a centrifuge at 2650 g. for 3 minutes (to remove surplus T.P. from the treated cells, B) and resuspended in isotonic saline. The cells were inoculated into batches of fresh mice (A) and (B). A further batch of mice (C) was inoculated with cultured cells which had been treated with T.P., centrifuged as above and re-suspended in their treatment medium. Thus, in column A in Table IV are shown longevities of the mice inoculated with cells which had no contact with T.P.; in column B are the corresponding figures for cells treated with T.P. for the length of time

TABLE IV.—*Survival of Mice in Days After Injection of T.P. Treated Ascites Tumour Cells*

Length of *in vitro* treatment	*A*	*B*	*C*
5 minutes	24·0	19·2	41·4
½ hour	15·4	24·4	33·2
5½ hours	17·2	80·8+	90·0+
24 hours	19·8	33·6	72·0+

EXPLANATION OF PLATES.

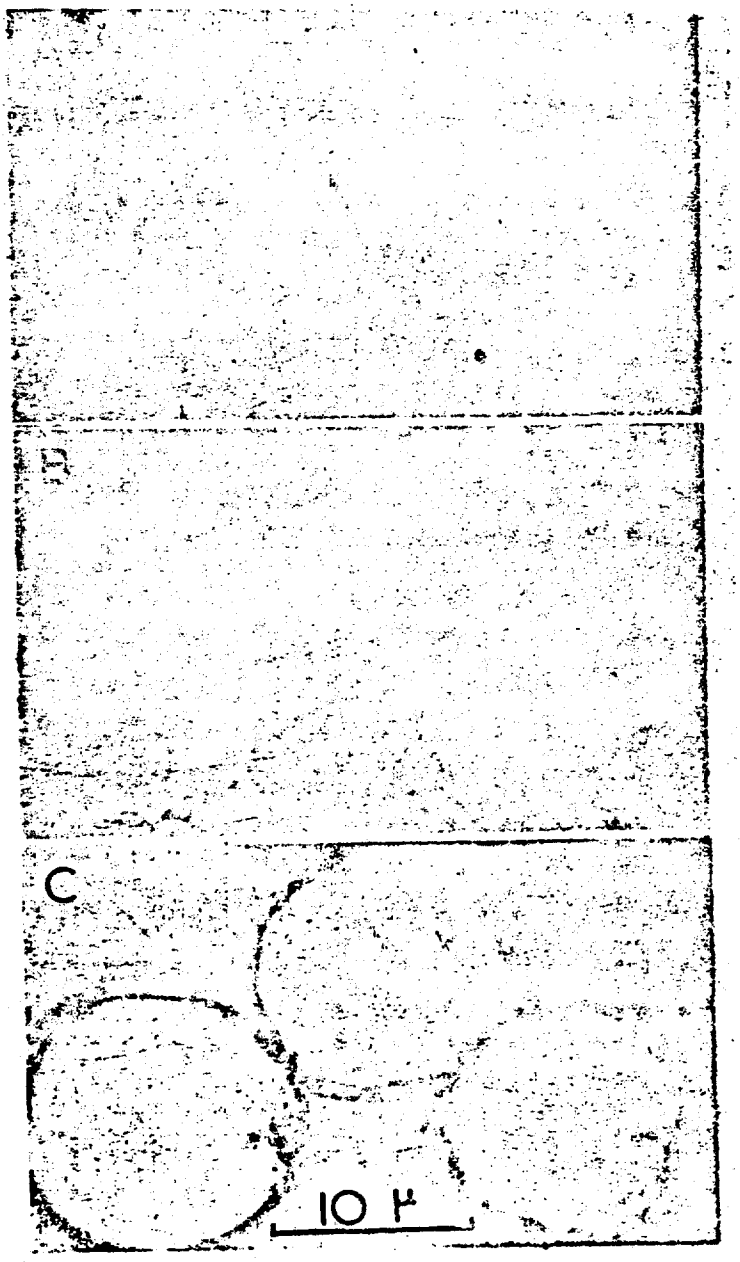
FIG. 1.—Aceto-orcein squash preparations of ascites tumour cells.

A. Control.
B. 24 hours after treatment with Tragacanth Powder, 2 mg./mouse.
C. 48 hours after treatment.
D. 72 hours after treatment.

FIG. 2.—Ascites tumour cells stained with Hotchkiss' stain for polysaccharides.

A. Control.
B. Treated for 1 hour with 1 mg./ml. Tragacanth Powder. Centrifuged and washed before staining.
C. As B, but not centrifuged or washed before staining.

A
C
D
d
l
10 μ



2

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given in the table; and in column C are results for cells still in contact with T.P. while in the peritoneal cavity.

Each figure is the average survival in days of 5 mice. The figures with + signs indicate that not all the mice in those batches were dead when the experiment was terminated.

Control mice (A) inoculated with cells kept in saline for 5 minutes lived longer than the other A mice because it was not possible to use the culture apparatus for such a short experiment, and the tumour was therefore not so well protected from temperature shock and infection. Similarly, in the other three batches of A mice length of life increased with length of *in vitro* cell culturing due to progressive deterioration of the culture. Nevertheless, by comparing columns A and B, it can be seen that T.P. treatment of ½ hour or more inhibited the tumour growth and increased the life of the mice, while 5 minutes' treatment had no effect. Column C mice showed greater longevity than B mice, since the treatment was continued intraperitoneally.

IV. Polysaccharide staining

Since the main constituent of T.P. is a mixture of polysaccharides (James and Smith, 1945; Hirst, 1951), the Hotchkiss stain for polysaccharides (Glick, 1949) would be expected to give a positive reaction. Normal, untreated ascites tumour cells stained by Hotchkiss' method after Carnoy fixation show only slight positive staining, which appears in the cytoplasm, and, in some cells, is concentrated in spherical cytoplasmic granules.

To test for polysaccharide in T.P. treated tumour cells seven-day-old ascites tumour was removed from mice and incubated with T.P. solution, 1 mg./ml. in physiological saline at 37° C., for different times. The concentration of cells in the suspension was $2{\cdot}0 \times 10^7$ cells c.c., and aliquots of the cell suspension were removed after incubation for 5 minutes, 30 minutes, 1 hour and 3 hours. Smears of these cells were then fixed in Carnoy, stained by the Hotchkiss method and examined under high power and compared with control tumour cells which had been incubated in saline alone. Further aliquots of the cell suspension incubated with T.P. were centrifuged, washed in saline to remove excess T.P. and fixed and stained as above.

Some results of these experiments are illustrated in Fig. 2. Adjacent to the cell membrane in cells incubated with T.P., positively staining material was found which was absent in the controls. The stained material seemed to form a layer on the cell membrane. In uncentrifuged cells the membrane appeared to be completely covered while in most of the washed cells the coating was reduced.

These results suggest that T.P. attaches itself to the cell membrane within the first half-hour of *in vitro* treatment. Even after centrifuging the staining remains visible showing that the binding is strong. Further experiments are in progress to detect any later penetration of the T.P. into the tumour cells, and any changes in their surface properties on treatment.

DISCUSSION

From the above results it appears that the immediate action of T.P. on the ascites tumour cell is to attach itself to the cell membrane. The dead cell counts show that this does not alter the cell permeability to the vital stain Lissamine

Green : and it is seen that control and treated cells " die ", i.e. become permeable to Lissamine Green, at the same rate. This indicates that the T.P. is not directly toxic to the cells. Belkin *et al.* (1959) obtained similar results when examining the damaging effects of various plant polysaccharides administered intraperitoneally to S37 mouse ascites tumours. Eosin was used as indicator in these experiments and treated and untreated tumours showed similar proportions of diffusely staining cells. Much more extensive vacuolisation of the cytoplasm was observed by these authors after treatment with the effective plant polysaccharides than in our experiments with T.P. It should be noted that T.P. (" commercial product ") was not effective in the experiments of Belkin *et al.* ; possibly the sample used was from a low-grade gum.

An increase in cell volume was also reported by these authors and this appears in our own preliminary experiments with medium T.P. doses (1 or 2 mg. in a 12-day-old tumour) although at higher doses (5 or 10 mg. in a 5-day-old tumour) cell shrinkage occurs. The changes in volume of the treated cells suggest an alteration in their permeability which is undetected by Lissamine Green tests and experiments are in progress to investigate the uptake of a different vital stain, i.e. the basic dye, acridine orange.

From the *in vitro–in vivo* experiments, it seems that T.P. acts on the cells within half an hour to reduce mitotic activity, while the histochemical staining shows that the polysaccharide component does not penetrate the cell in this time. Of course, non-staining constituents of the T.P. may penetrate the cell, or it may decompose slowly at the cell surface and its products may enter the cell. Alternatively, it is possible that T.P. constituents coating the cell prevent intake of essential nutrients or exit of toxic products of cell metabolism. Related experiments have been reported recently by Kornguth, Stahlmann and Anderson (1961) using a fluorescent derivative of the basic polypeptide polylysine. This was incubated with Ehrlich ascites tumour cells, against which it shows a growth-inhibitory effect *in vivo*. Very little, if any, of this polypeptide appeared to enter the cells within the time of the experiment (10 minutes), but most of the material was observed bound to the cell surface in fluorescent clumps. It is possible that in experiments such as these the different polyelectrolytes are distinguishing different areas of specific charge on the cell membrane.

However it may occur, the net result of T.P. treatment of the ascites tumour cells is the suppression of mitosis, probably as a direct effect during interphase or early prophase which manifests itself in the prophase counts. Very few metaphase nuclei are found. The cells blocked during division degenerate into a pycnotic state and later disintegrate. Increase in white blood cell counts possibly occurs as a response to the breakdown products of the tumour cells, although Belkin *et al.* (1959) also noted a pronounced intraperitoneal leucocytosis after injection into the Sarcoma 37 ascites tumour of their T.P. sample, which did not cause cell swelling and vacuolisation.

SUMMARY

Tragacanth Powder (T.P.) inhibits ascites tumour growth in mice. Evidence is put forward to show that T.P. becomes attached to the cell membrane, and that it acts as a mitotic block, probably indirectly, the direct effect occurring in the interphase or early prophase cell.

The authors are grateful to Professor A. Haddow, F.R.S., for his encouragement in this work.

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THE ALLERGENIC PROPERTIES OF THE VEGETABLE GUMS

A Case of Asthma Due to Tragacanth

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REPORTS of sensitivity to various commercial gums have appeared increasingly during the past decade, with gum arabic (acacia) and karaya most often found to be the causative agents. The usual mode of entry is by inhalation, but ingestion and surface contact also have accounted for some cases. The symptoms include vasomotor rhinitis, bronchial asthma, urticaria, atopic dermatitis, angioneurotic edema, and gastrointestinal disturbances. In a large majority of cases sensitization occurs through occupational contact with the gums, and clinical symptoms arise only after a considerable period of exposure. In order to show the wide distribution of these allergens and the conditions under which they may be encountered, the reports thus far published will be briefly reviewed.

I. Review of the Literature

Acacia (Gum Arabic).—Experimental work with animals by Maytum and Magath[11] (1932) showed acacia to be mildly antigenic. It caused no reactions in rabbits, but varying degrees of anaphylaxis could be induced in about 63 per cent of the guinea pigs tested. Despite these findings, however, and the low nitrogen content of gum arabic, it has been shown to cause severe clinical symptoms in a large number of persons. The above-mentioned authors observed a patient under treatment for elephantiasis of the leg, who, following a Kondoleon operation, received 500 c.c. of a 6 per cent solution of acacia and 500 c.c. of physiologic saline solution intravenously. No untoward symptoms occurred at the time, but seven months later, when the therapeutic injection was repeated following a second operation, the patient developed nasal obstruction, lachrymation, loss of voice, coughing, and a suggestion of laryngeal stridor. These manifestations were mild and easily relieved by epinephrine. The patient had shown no previous signs of allergy, but her family history was positive.

Much more severe reactions to the intravenous injection of acacia were reported by Studdeford[15] (1937). In three patients suffering from post-partum hemorrhage, infusions of acacia glucose solution produced acute constitutional reactions marked by cyanosis, dyspnea, tachycardia, and pulmonary edema. Two of the patients died, and autopsy upon

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one disclosed an extensive destructive lesion of the liver. Believing that impurities in the acacia preparations might have caused these untoward effects, the author tried fresh material from another supply house, but this produced similar results in three other patients, one of whom died.

Studdeford observed that recent experimental work has shown the likelihood of liver damage following the intravenous injection of acacia. It seriously disturbs the red blood cells, interfering with the normal gaseous interchange, increasing the tendency to rouleaux formation, and accelerating the sedimentation rate. Conglutination of red cells may occur, producing blockage followed by edema and hemorrhage.

Experiments on dogs by Hall, Gibson, and Weed[7] (1940) showed that repeated intravenous injections of the gum damaged the carbohydrate and serum protein functions of the liver, as evidenced by changes in the glucose and galactose blood sugar tolerance curves and determinations of plasma proteins. However, no effect was observed on the hepatic cells, nor was there any blocking of the reticulo-endothelial system comparable to that produced by some colloidal substances.

Spielman and Baldwin[14] (1933) described a case of acacia sensitivity in a plaster molder employed in a candy factory. He developed vasomotor rhinitis and bronchial asthma after working about six months in the plant. Direct tests produced a marked reaction to the factory dust containing crude acacia and also to purified acacia. Passive transfer tests likewise were positive.

Allergy to acacia among printers appears to be growing more and more frequent. The gum is used in solution with dextrin, alcohol, and water as a drying, or offset, spray for printed material. The spray fills the workrooms with a fine mist which the employees unavoidably inhale unless protected by a mask.

Feinberg and Schoenkerman[5] (1940) reported a case of bronchial asthma in a printer which they attributed to this cause. The patient gave positive skin reactions to gum arabic and also to karaya. Following this, Bohner, Sheldon, and Trenis[1] (1941) published ten similar cases. All their patients reacted to gum arabic by direct tests, and passive transfer was positive whenever tried. These printers had been exposed to the acacia solution for periods of from two weeks to a year. The authors state that "the direct transfer tests were negative to Indian gum and tragacanth" ("Indian gum" probably referring to karaya).

King[9] (1941) treated one case of bronchial asthma and another of vasomotor rhinitis in printers, both of whom were positive to acacia. These patients were of allergic constitution and reacted to other substances, such as orris root, egg white, house dust, and pollen, but clinical symptoms did not appear until they became sensitized by gum arabic in their occupation. Attempts at desensitization in one patient were unavailing while he remained in his job. King refers to the New

York State Department of Labor Bulletin for 1940,[13] which also mentions a number of cases of rhinitis and asthma among printers, probably due to gum arabic.

According to our search through the literature, Levin[10] (1939) appears to have been the first to record a case of sensitization to acacia in the printing trade. It probably is only in recent years that this type of offset spray has been brought into use.

Feinberg and Schoenkerman[5] described the case of a furniture dealer with asthma of many years' standing, which probably was due to infection. Nevertheless, the patient gave a marked skin reaction to acacia, which proved to be an ingredient in some of his furniture preparations. Desensitization with the gum appeared to afford some temporary relief.

Karaya Gum.—Bullen[3] (1934) was the first to report a case of allergy to karaya. His patient developed vasomotor rhinitis from contact with the gum in hair-waving lotion. Since that time such reports have become quite numerous. Feinberg[4] (1935) observed bronchial asthma in a hairdresser from the same cause. Scratch tests with powdered karaya gum, sodium benzoate, tragacanth, acacia, and another brand of hair-waving fluid all were negative except that with karaya, which, he said, produced an "enormous reaction."

Bowen[2] (1939) reported five cases of urticaria due to karaya. One patient had associated respiratory symptoms. He gave details of one case in which the urticaria was accompanied by severe pruritus and some lesions of angioneurotic edema, frequently involving the joints. The edematous lesions were succeeded by patches of hyperpigmentation. This patient's symptoms were traced to karaya gum in Dr. Wernet's dental powder, used for holding her denture in place. She gave no family history of allergy.

Figley[6] (1940) observed sixteen women with allergic symptoms, all of whom gave positive reactions to scratch tests with karaya. The family history was positive in all but two. The allergen was absorbed by ingestion and surface contact as well as by inhalation, and the chief manifestations were perennial rhinitis, asthma, atopic dermatitis, urticaria, and gastrointestinal distress. Wave lotions, laxatives, emulsified mineral oil, gelatins, diabetic foods, tooth pastes, and denture adhesive powders were the sources of sensitization.

In investigating the effect of Mucara, a laxative containing karaya, Ivy[8] found that 7.8 per cent of eighty-nine test subjects who ingested this preparation complained of mild abdominal cramps or other discomfort for which he could not account. Figley suggested the possibility that these patients were slightly sensitive to karaya through having previously ingested it in some food or confection.

Feinberg and Schoenkerman[5] analyzed ten cases of allergy to karaya resulting from contact with hair-waving lotions or powders. Respiratory

symptoms were the chief complaint in nine of the patients, one of whom had an associated dermatitis of the face and scalp. The tenth complained only of dermatitis of the face and neck. Cutaneous reactions to karaya were consistently positive in all the patients by direct test and also by passive transfer when this was performed. On being tested with other gums, in accordance with the authors' established routine, seven patients gave negative reactions, two reacted slightly to tragacanth alone, and one to acacia and tragacanth. The authors believed karaya to be the sole cause of clinical symptoms in four of the patients and a contributory factor in the remainder. In one patient with bronchial asthma, desensitization with karaya produced moderate improvement; in another, a change to tragacanth preparations proved beneficial.

The most common ingredients in hair-waving lotions are acacia, tragacanth, linseed gum, quince seed gum, karaya; boric acid, sodium, potassium, and ammonium carbonate; alcoholic keratin, coloe, petrolatum, cera-flux, glyco wax A, and paraflux. According to Figley, however, karaya has almost supplanted such gums as linseed and quince seed, and this fact may account for the growing number of allergic manifestations reported from contact with these preparations.

Gum Tragacanth.—Reports of sensitivity to tragacanth are extremely rare in the literature. One case has been described by Feinberg and Schoenkerman, in which a hay fever patient suffering from severe urticaria and eczematous dermatitis of the hands gave a marked reaction to tragacanth. The authors believed her cutaneous symptoms to be due to a hand lotion containing this gum but were unable to follow the case closely enough to obtain conclusive proof. We already have mentioned that three of their karaya-sensitive patients gave slight reactions also to tragacanth. They stated that they were unable to find any cases in the literature that incriminated tragacanth as an allergen, but "because of its close relation to karaya gum," they employed it in routine testing.

Owing to its comparative rarity, therefore, the following history* may be of particular interest. We believe it to be the first thoroughly authenticated case of clinical allergy caused by sensitivity to tragacanth.

B. T., white, female, aged 26 years, came to Gouverneur Hospital on July 7, 1941, to ask for desensitization to certain commercial gums which she believed to have caused her respiratory symptoms. She gave the following history:

In December, 1939, she went to work in the office of a New York gum factory. This firm imports various commercial gums from the Orient and mills them on the upper floor of its office building. The material is passed from one floor to another through a chute which is not airtight,

*This case report was presented before the Associated Allergy Clinics of Greater New York at their fall meeting, Nov. 6, 1941. The presentation was made by Dr. Maury D. Sanger and discussed by the author.

and office workers as well as the millers are exposed to dust given off by the gums. The patient's desk was situated close to the chute, samples of the material were handled by her, and other samples were exposed in open containers on the office floor. Moreover, she frequently came in contact with the mill workers.

When the patient began to work in this place she was in perfect health and remained so for about a year. Then, in December, 1940, she became troubled with what she supposed to be a persistent head cold, with blocking of the nose, frequent sneezing, and profuse nasal discharge. She began to feel "run down" and decided to remain at home to recuperate. On a brief visit to the factory on New Year's Day, 1941, however, she apparently contracted a "fresh cold." After her return to work this also persisted, and by the end of February, fourteen months after her first contact with the gums, she began to cough and wheeze.

On March 3, 1941, fifteen months after first exposure, and coincident with the milling of an unusual amount of tragacanth, the patient was seized with a severe attack of asthma, lasting for eight hours, and finally was relieved by epinephrine.

From that time on, up to June 20, 1941, similar attacks occurred whenever she returned to her job after sick leave or vacations. All were relieved by epinephrine. After an extremely severe seizure on June 20, she resolved never to return to the factory. But, in order to prove that the gums were responsible for her trouble, she tried various temporary positions in other kinds of business. In the new surroundings, no untoward symptoms occurred.

Convinced now that she was allergic to the gums, and her return to the job being greatly desired, the patient came to our clinic to be desensitized.

Personal and family history, as given, were completely negative. Physical examination revealed no sign of infection in the nasopharynx or sinuses. X-ray examinations of the chest and sinuses also were negative.

Direct skin tests with the usual allergens produced no response, but when gum tragacanth, 100 units, was tried, a marked reaction occurred. Gum arabic, 1,000 units, also produced a strong reaction. Passive transfer tests with these two gums were strongly positive down to the 1:100 serum dilution. On the other hand, direct tests with karaya up to 5,000 units failed to provoke any response.

Immunization treatment with tragacanth was attempted with great caution for fear of untoward reactions, and the dosage increased at first only from 5 to 10 units for each successive treatment.

After receiving thirty injections, the patient believed herself to be immunized and decided to revisit the gum plant. Upon only a half-hour exposure, however, she experienced an ominous tightness in the chest.

TABLE I

OCCUPATIONAL ALLERGY IN A GUM FACTORY

CASE NO.	NAME	SEX	AGE (YR.)	TYPE OF WORK	TIME EMPLOYED	ALLERGIC SYMPTOMS	REACTION TO TESTS			REMARKS
							KARAYA	GUM ARABIC	TRAGACANTH	
					Office Workers					
1	M. W.	M	63	Manager	30 yr.	None	Neg.	Neg.	Neg.	--
2	V. L.	M	25	Chemist	4 yr.	None	Neg.	Neg.	Neg.	--
3	M. K.	F		Clerk	1 yr.	Rhinitis, asthma	--	Mkd.	--	Left job
4	B. T.	F	27	Secretary	1 yr.	Rhinitis, asthma	Neg.	Mkd.	Mkd.	Left job
					Factory Workers					
5	M. B.	M	42	Packer	11 yr.	Nasal clogging, dyspnea	Neg.	Mod.-Mkd.	Neg.	--
6	G. S.	M	35	Miller	2½ mo.	Bronchial asthma	Neg.	Neg.	Neg.	Asthma due to infection
7	L. O.	M	33	Miller	12 yr.	None	Neg.	Mod.-Mkd.	Neg.	--
8	J. P.	M	21	Elevator operator	1½ mo.	None	Neg.	Neg.	Neg.	--
9	J. C.	M	39	Laborer	15 yr.	None	Neg.	Neg.	Neg.	--
10	E. W.	M	38	Shipping clerk	4 yr.	None	Mod.	Mod.-Mkd.	Neg.	--
11	A. O.	M		Foreman	5 yr.	Nasal clogging, sneezing	Neg.	Mod.-Mkd.	Mod.	--
12	J. S.	M	22	Miller	1½ yr.	Sneezing, dyspnea	Mod.	Mod.-Mkd.	Mkd.	--

Summary

	NUMBER EXAMINED	NUMBER AFFECTED	NUMBER NOT AFFECTED	CAUSE OF SYMPTOMS		POSITIVE SKIN REACTIONS		
				GUMS	INFECTION	KARAYA	GUM ARABIC	TRAGACANTH
Total	12	6	6	5	1	2	7	3
Office workers	4	2	2	2	0	0	2	1
Factory workers	8	4	4	3	1	2	5	2

On reaching home she was seized with an exceedingly severe attack of bronchial asthma. A number of injections of epinephrine were required before the paroxysms subsided, and she remained ill for a week. This experience caused the patient to abandon all hope of returning to her job, and she also discontinued treatment.

II. SENSITIZATION TO GUMS AS AN OCCUPATIONAL RISK

The striking character of the foregoing case and the excellent field for research provided by a factory solely concerned with the handling and processing of allergenic gums led us to study the incidence of sensitivity among the other employees.

We found that the firm had been in business for more than a century, and during that time certain employees in the mill (formerly in a separate building) had had symptoms of vasomotor rhinitis and severe bronchial asthma after contact with tragacanth, gum arabic, and karaya. In most cases, the affected workers had to leave their occupation, but some were able to carry on, and, in time, appear to have become, so to speak, immune.

In recent years the milling has been done in the same building with the executive offices, and we learned that another female office worker, previous to our survey, had been obliged to give up her work on account of nasal symptoms and bronchial asthma, coming on about a year after first exposure. Her physician had found her to be sensitive to gum arabic.

In order to gain more information as to the sensitizing properties of the gums and their antigenic relationships, we carried out a study among both the office and mill workers. We were unable to examine the entire personnel, but those who were willing to undergo tests made up a good cross-section.

No further complaints were discovered among the office force, but investigation of the millers revealed a somewhat different picture. Most of the men had been in the mill for a number of years under conditions of massive exposure, and the factor of mechanical irritation as well as prolonged contact was involved. Nevertheless, many of them had escaped untoward symptoms. Table I shows the results of our examinations thus far.

It will be noted that 50 per cent of all the workers examined had respiratory symptoms of an allergic nature. Among the four office employees, one* was sensitive to gum arabic and another to gum arabic and tragacanth but not to karaya. These workers had to leave their jobs. Of the eight mill workers, three had nasal clogging and sneezing (with or without constriction of the chest) when in contact with certain gums, and these three were found sensitive to the materials by direct

*This refers to M. K., who had left the plant previous to our survey. However, since we were able to obtain her history, it has been included.

skin tests. One had asthma traceable to infection in the sinuses and unrelated to his occupational contacts.

Comment.—In view of the contradictory findings in a few of these cases, the question arises—how many of the men who gave positive skin tests, but denied having symptoms, were concealing their complaints for fear of endangering their jobs? With one or two of them we had a strong impression that this was the case. Nevertheless, the fact remains that a certain number of employees definitely remained free from untoward symptoms and had no signs of clinical pathology discoverable by physical or roentgen examination or by skin tests. Those who did acknowledge allergic symptoms, with one exception, also gave positive skin reactions to one or more of the gums. Hence, it is clear that irritation and prolonged contact with tragacanth, gum arabic, and karaya affect some persons but fail to affect others working under the same conditions. We must conclude that the individuals who become sensitized have an inherent predisposition to allergy, which probably is hereditary, although no family history of hypersensitiveness could be elicited in any of these cases.

The case of B. T. is the most clear-cut of any that came under our observation and the most enlightening. First, it demonstrated the fact that gum tragacanth as well as gum arabic is a sensitizing agent capable of producing severe respiratory symptoms in predisposed individuals. Second, the patient was found, by both direct and passive transfer tests, to be sensitive to gum arabic and tragacanth but not to karaya, despite the fact that the antigenicity of karaya has been so well established. This tends to disprove the general assumption that all these gums are closely related. Third, it was shown that a period of time was required after first contact to bring forth the acute sensitivity. Fourth, since the treatments though limited were supplemented by long absence from exposure and yet failed to confer any degree of immunity, desensitization probably would be unavailing.

III. ANTIGENIC RELATIONSHIP OF THE GUMS

The general impression has been that the three gums under consideration are closely related both antigenically and botanically, but few observers appear to have carried out in vitro and in vivo neutralization experiments with the sera of patients known to be clinically sensitive to them. Since our clinical findings in the cases of B. T. and A. O. seemed to disprove the theory of a common antigen in karaya and the other two gums, we decided to make such studies in order to determine the antigenic relationship of tragacanth and gum arabic.

Sera were obtained from all the workers found to be sensitive, and all except one gave a positive passive transfer. For the neutralization studies, however, only one serum, that of B. T., remained suitable for study. A few hemolyzed, and the patients refused to give more blood. In one case the Wassermann was doubtful.

Following is the result of our studies with B. T. serum:

Exhaustion-Site Studies (Figs. 1 and 2).—

Method: Duplicate sites in nonatopic recipients were passively sensitized by injecting 0.1 c.c. of B. T. serum, which was clinically sensitive to gum tragacanth and gum arabic. The next day, when the reagins were fixed in the tissues, the first site (above) was tested with

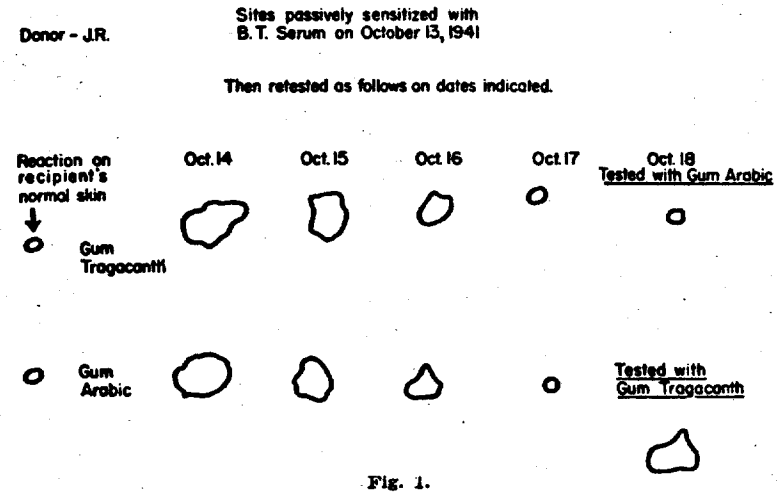

Fig. 1.

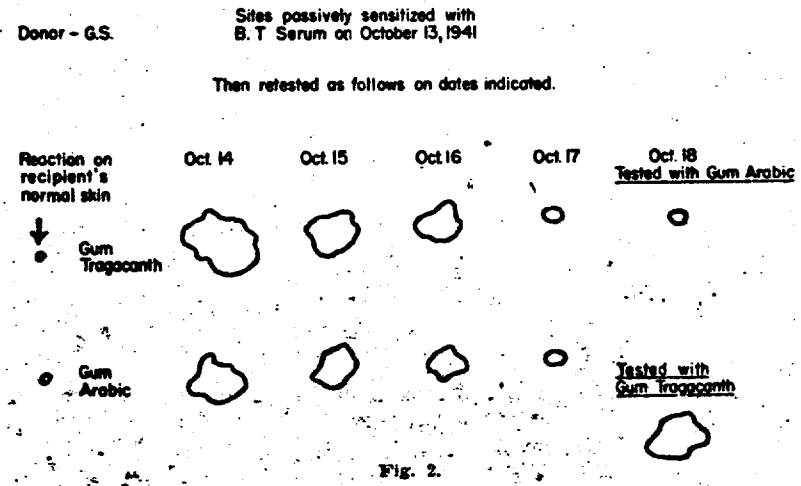

Fig. 2.

gum tragacanth and then retested daily until no reaction occurred. The following day the site was tested with gum arabic. The second site was desensitized in the same way to gum arabic and then tested with tragacanth.

Results: In both donors the reagins to gum arabic were completely neutralized by tragacanth, but gum arabic failed to neutralize the reagins to tragacanth.

In Vitro Neutralization Studies (Figs. 3 and 4).—

Method: Having previously determined that the immediate and forty-eight-hour tests showed neutralization at 500 to 700 units,* we prepared in the test tube under sterile precautions a mixture of two parts extract (0.2 c.c. of 5,000 units, representing 1,000 units) to one

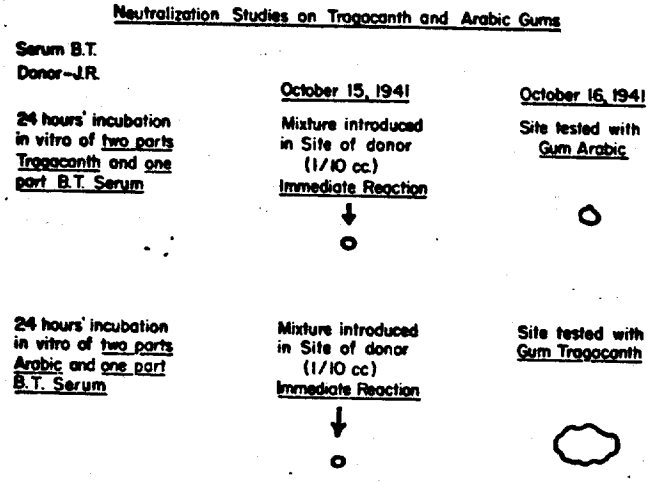

Fig. 3.

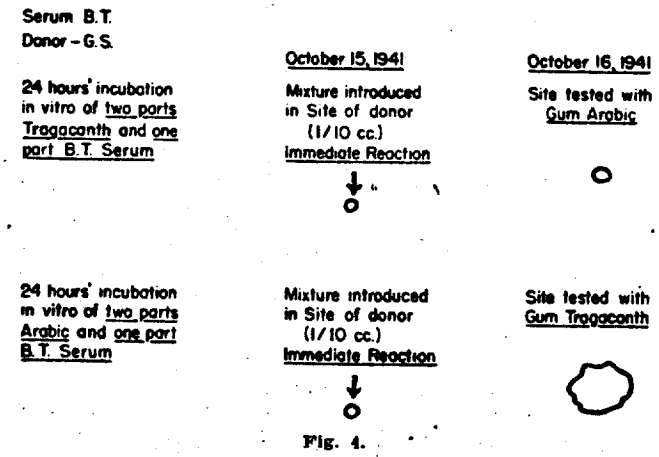

Fig. 4.

part serum (0.1 c.c.) for each gum, tragacanth and arabic. After being allowed to stand in the icebox for twenty-four hours, each mixture was injected in 0.1 c.c. amounts into duplicate sites on nonatopic subjects.

*This was done by mixing in the test tube increasing amounts of antigen with a fixed amount of sensitive serum, allowing it to incubate in the icebox, and then testing test amounts into a nonatopic donor. The immediate reaction was observed, and 48 hours later each site was tested with 1,000 units of the gum antigen, and the neutralization point was noted

Twenty-four hours later the sites injected with serum neutralized to tragacanth were tested with gum arabic, and vice versa. Duplicate control sites, having been prepared with two parts physiologic saline solution and one part serum, also were tested with each gum.

Results: Here, too, we found that tragacanth neutralized the reagins to itself as well as to gum arabic, but arabic, while neutralizing its own reagins, failed to neutralize the reagins to tragacanth.

Comment.—The direct skin tests in the case of B. T. suggested a probable antigenic relationship between tragacanth and arabic, but none between either of these gums and karaya. The serologic studies both in vivo and in vitro confirmed the relationship between the two gums to which the patient was clinically sensitive, since it was shown that gum tragacanth completely neutralized the reagins to gum arabic, but that gum arabic failed to neutralize all the reagins to tragacanth. It was noted that tragacanth and arabic extracts were similar in nitrogen content but that direct skin tests on this patient elicited a greater reaction to tragacanth. The impression was thus gained that tragacanth extract either contains an antigen not present in gum arabic or that it is richer than gum arabic in atopic excitant content.

IV. NATURE AND DERIVATION OF ALLERGENIC GUMS

This is a subject on which the information from various authoritative sources long has been indefinite and conflicting. Medical knowledge is therefore in a state of confusion, and it is small wonder that statements appearing in the literature show many contradictions.

We have consulted different authorities and have obtained the following information from the United States Dispensatory for 1937:

The term gum, often loosely applied to the resinous exudate of plants, refers more correctly to exudates which contain a carbohydrate that is capable of forming with water mucilaginous mixtures. The belief that gums differ from resins in being soluble in water and insoluble in alcohol is incorrect. Some gums do not actually dissolve in water but simply swell into viscid mixtures with it, and some gums are soluble in alcohol.

The gums that are most often considered identical, or whose names are frequently confused with one another, are described as follows:

Bassora Gum (Caramania Gum, or Hog Gum): Bassora gum is a collective term for a group of high-colored gums somewhat resembling tragacanth. The soluble part is arabin and is said to constitute about 11.2 per cent. The insoluble part consists of bassorin, with a small proportion of saline substances. The gum is used as an adulterant for acacia and can be distinguished by its insolubility in water. Its botanical origin is doubtful.

Karaya Gum: Karaya gum is known as Sterculia gum or Indian tragacanth. It is derived from *Sterculia urens* Roxb. (Fam. Sterculiaceae), of Asia, and possibly other species of Sterculia. It is found in irregularly shaped, pinkish brown or light

brown pieces and has an acateous odor. When boiled with 5 per cent potassium hydroxide solution, it shows only a slight tinge of brown, whereas tragacanth thus boiled becomes a bright yellow and gives a stringy precipitate. Tragacanth is more mucilaginous than karaya, and karaya has greater acidity (due to acetic acid) and dissolves more readily in cold water. Nevertheless, powdered karaya often is used as a substitute for powdered tragacanth and loosely *called* tragacanth.

India Gum (Ghatti Gum): The term India gum has been applied to many different substances, including Bassora gum, Sterculia gum, and Ghatti gum (Gummi indicum). The British Pharmacopoeia in 1914 recognized Ghatti gum as India gum. It is derived from different species of the Indian tree *Anogeissus latifolia* (Fam. Combretaceae). It is used in pharmacy for the same purposes as acacia. Its mucilage is more viscid but less adhesive than acacia and is usually employed in connection with tumeric, with which there is some specific combination.

Gum Arabic (Acacia): Gum arabic comes from the stems and branches of *Acacia senegal* Willdenow (Fam. Leguminosae), or some other African species of Acacia. It is whitish yellow or light amber in color and is insoluble in alcohol but almost completely soluble in water, the solution being acid. It is composed essentially of the calcium salt of arabin, or arabic acid. The gum is an effective demulcent and is extensively used in drugs, pills, and lozenges.

Gum arabic often is adulterated with Mesquite gum, from a Mexican plant, *Prosopsis juliflora* (Fam. Leguminosae).

Tragacanth Gum: This substance is an exudation from *Astragalus gummifer* Labillardière (Fam. Leguminosae) and other Asiatic species of Astragalus. In the past there has been much doubt as to its botanical source, but it now is known to be of the above genus and family. It is entirely insoluble in alcohol and seems to be composed of two different constituents, one being soluble in water and resembling gum arabic, and the other swelling in water but not dissolving. The soluble part, which is much the greater, is said by A. G. Norman (1931) to consist of uronic acid and arabinose in about equal proportion, which compose 94 per cent, plus small amounts of cellulose, starch, and protein substances. The insoluble part is mostly bassorin.

Tragacanth is sometimes adulterated with Sterculia (karaya) and Ghatti gum but more frequently with acacia. The difference between true tragacanth and Sterculia can be proved by a distillation test with water and determination of the acidity, which is greater in Sterculia.

According to Chemical Abstracts (1939), tragacanth contains enough starch to give a blue color with iodine solution, but acacia contains no starch. The methoxyl ($CH_3.O$—) indexes of the three gums under consideration are: acacia, 0-12.4; tragacanth, 18.6-38; karaya (Sterculia), 0.

Figley[6] quotes Norman (1929) to the effect that no essential difference exists between gums and hemicellulose; in both, hexose and pentose are linked with uronic acid. He also quotes Solis-Cohen, who states that arabin, bassorin, and cerasin are the proximate principles of gums, and that gums are chiefly pentosans.

Bohner, Sheldon, and Trenis[1] say that acacia is classed chemically as an inert colloid and is considered to be a polysaccharide member of the carbohydrate family, related polysaccharides being glycogen, dextrin, and starch. All of these are amorphous, odorless, and translucent, and on hydrolysis yield one or more sugars, usually pentoses and hexoses.

In discussing Bullen's paper,[3] Baldwin said the fact that gums are listed as polysaccharides is misleading, because in the case of acacia, 0.5 per cent of total nitrogen is present and it also gives a positive biuret test. In an analysis of three samples purchased in the open market, Bohner and his associates found an average of 0.48 per cent of nitrogen and a positive biuret test. They note that Uhlenhuth and Remy[16] found 0.3 per cent of nitrogen in samples of purified acacia.

Figley[6] stated that the nitrogen content of karaya is only 0.1 per cent.

V. SOURCES OF CONTACT WITH ALLERGENIC GUMS

We have compiled the following list from data gathered by many of the authors quoted in this paper and from other available sources. Since we know that the various gums frequently are substituted for one another and adulterated by one another and by other gums, no attempt at separate classifications has been made:

Adhesive pastes
Artificial flowers
Body and drier in lithograph inks
Candy (e.g., gumdrops and jellies)
Cement
Cheese
Cigar manufacture
Coating for special thread
Custards (e.g., in factory-made pasteries, etc.)
Denture adhesive powders (Dr. Wernet's, Dent-A-Firm, Stix)
Diabetic foods (e.g., soy bean and almond wafers)
Emulsions (e.g., mineral oil, cod-liver oil, turpentine, almond, and flavor emulsions)
Fireworks
Furniture polishes
Gelatines
Glues
Ice cream mixes
Insecticides
Laxatives (e.g., Imbicoll, Mucara, Squibb's Petroleum and Agar)
Linoleum and oil cloth
Lotions (cosmetic, for hand care, hair-waving, etc.)
Luster for textiles
Match manufacturing
Metal polish manufacturing
Mucilages
Paints
Pills
Porcelain and pottery manufacture
Printing ink manufacture
Process engraving
Salad dressings (factory made)
Shoe polishes
Sizing of paper and textiles
Sprays (offset in printing trade)

Starch (special)
Suppositories
Toothpastes (Listerine and Lactora)
Textile printing
Vaginal jellies
Varnishes
Water colors (transparent)

No doubt these gums also occur in many other preparations in which their presence has not yet been disclosed, and the sources of contact probably are even more widespread than the above list would indicate.

SUMMARY

1. The published reports of sensitivity to vegetable gums have been reviewed. These reveal that the chief sources of sensitization are karaya gum in hair-waving lotions and acacia in the offset sprays used in the printing trade. Although the allergens may enter the system by ingestion, injection, or surface contact, inhalation is the most common route, and respiratory symptoms predominate.

2. The first well-established case of sensitization to tragacanth is reported. This case led to a study of the incidence of gum sensitivity among the workers in a gum factory, which has brought forth the following facts: (a) that gum sensitization is an occupational risk for predisposed persons; (b) that a period of time (usually about a year) is required after first exposure for the development of acute symptoms; (c) that tragacanth is a powerful allergen capable of causing extremely severe reactions; (d) that desensitization with allergenic gums is difficult, if not impossible; (e) that some workers who become sensitized may afterward spontaneously develop tolerance.

3. The antigenic relationship between gum arabic and tragacanth has been studied by means of in vivo and in vitro experiments with sensitive sera. It was found that tragacanth is able to neutralize all the reagins to gum arabic but that arabic only partially neutralizes the reagins to tragacanth. Thus it appears that, while an antigenic relationship probably exists between these gums, and although the nitrogen content may be the same in both extracts, tragacanth must either contain an antigen not present in arabic or its extract must be richer in excitant content. The relationship between karaya and the other gums was not studied.

4. Consultation of various authorities on the botanical origin of the three gums revealed that gum arabic (*Acacia senegal*) and tragacanth (*Astragalus gummifer*), although differing in genus and species, are members of the same family (Leguminosae). Karaya, on the other hand, belongs to an entirely different family (Sterculiaceae). This fact may have some bearing on the antigenic relationship of the gums as brought out in our studies.

It is interesting that Dr. Gelfand's twelve patients studied were presumably equally exposed by inhalation to the three gums; that the greatest number (six) reacted to gum arabic; that tragacanth was next with three reactors; and karaya last with two reactors. May one assume from this that arabic is the most potent allergen of the three?

DR. HOWARD OSGOOD, Buffalo, N. Y.—I have been testing routinely for some time with five different gums but have done no passive transfer studies. Just before leaving for this meeting, I pulled out at random the records of 100 allergic patients and summarized the results of the tests with these gums. I thought it might be of interest in connection with the present paper.

The gums used were acacia, tragacanth, quince seed, karaya, and ghatti. The latter is sometimes substituted for karaya in what is known as India gum. The concentrated extracts were 1:33 or 1:100 of the dry gum in Coca solution, depending on the ease of Seitz filtration. A 1:10 dilution was made up from the concentrated extract, and tests were carried out in each patient with both strengths.

Of the 100 patients tested, there were only two who failed to give skin reactions in some degree (slight, moderate, or marked) to one or more of the gums, in weak or strong dilution. There were twelve patients who reacted to one gum only, twenty-two who reacted to two only, twenty-two to three gums, fourteen to four, and there were twenty-eight patients who reacted in some degree to all five gums.

To determine the relative skin-exciting properties of the different gums, or conversely the relative skin reactivity of this group of 100 patients to the different gums, I have tabulated the maximum reaction given by each patient to each gum, without indicating to which strength (concentration or 1:10 dilution) the maximum reaction occurred. For example, to acacia thirty-four patients gave only a slight reaction to either dilution, thirty-three gave a moderate reaction, and twelve gave a marked reaction to one or the other dilutions. Ghatti gum gave the most numerous reactions, ninety-three patients reacting to some degree. Karaya gum with only twenty-five patients reacting, gave the fewest.

TABLE I

MAXIMUM DEGREE OF SKIN REACTIONS TO EACH GUM

	NUMBER OF PATIENTS GIVING				
	SLIGHT ONLY	MODERATE ONLY	MARKED	TOTAL	MODERATE OR MARKED
Acacia	34	33	12	79	45
Ghatti	42	36	15	93	51
Karaya	25	6	5	36	11
Tragacanth	26	12	10	48	22
Quince seed	42	16	4	62	20

I tried to see what cross reactions there might be to these five gums and have tabulated the frequency of associated reactions in single patients. Moderate and marked reactions only were considered. Eighteen gave moderate or marked skin reactions to acacia and ghatti gums and to no others, this being the most frequent association. The number of other patients giving reactions to two gums only showed the following associations: acacia-tragacanth, 2; acacia-quince, 2; ghatti-karaya, 2;

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ghatti-quince, 1. The number of patients showing reactions to three gums only gave the following associations: acacia-ghatti-quince, 9; acacia-ghatti-tragacanth, 5; ghatti-tragacanth-quince, 1. Eight patients gave moderate or marked reactions to four or to all five gums. It will be seen that the most frequent association of moderate or marked reactions occurred with acacia, ghatti, and quince seed.

I am presenting this statistical summary for what it may be worth and draw no definite conclusions. I am not ready to state that these positive skin reactions indicate clinical sensitivity to gums, although in a small number of these 100 patients this was definitely the case.

My own feeling is that in the dilutions used, the gum solutions may be intrinsically slightly irritating, thus explaining the numerous slight and moderate reactions. Typical marked reactions occurred in forty-six patients, most frequently to ghatti, acacia, and tragacanth. My second opinion is that there may be common allergens in some of the gums.

I have not delved into botanical relationships, as has Dr. Gelfand, but from his results, and from my meager observations, it would seem that further work with these gums along the lines laid out by Dr. Gelfand would be well worth while.

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(Continued)

THE VEGETABLE GUMS BY INGESTION IN THE ETIOLOGY OF ALLERGIC DISORDERS*†

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THE vegetable gums as causative factors in allergic disturbances, by means of inhalation, surface contact, and injection, have been recorded by various investigators[1-7] including the present writer.[11] The present paper stresses the importance of these gums as agents that cause allergic symptoms by ingestion.

Ivy and Isaacs' report[8] of the effect of the laxative Mucara which contains karaya should be mentioned here. In a group of eighty-nine test subjects, 7.8 per cent complained of mild abdominal cramps or other discomfort after the ingestion of this laxative.

Figley[9] observed sixteen women with allergic symptoms, all of whom gave positive reactions to scratch tests with karaya. The family histories for allergy were positive in all but two of these subjects. The allergens had been absorbed by ingestion and by surface contact, as well as by inhalation. The chief manifestations were perennial rhinitis, asthma, atopic dermatitis, urticaria, and gastrointestinal distress. Wave lotions, laxatives, emulsified mineral oil, gelatins, diabetic foods, tooth pastes, and denture adhesive powders were the sources of sensitization.

The first case report of an allergic reaction by ingestion, to drug tablets containing the vegetable gums, was presented by Brown and Crepea.[10] This was a white man who manifested symptoms of asthma and generalized urticaria from the ingestion of Pyribenzamine tablets which contain tragacanth. Skin tests, passive transfer, and clinical trial established this vegetable gum as the cause.

Further proof of the role of the vegetable gums, by ingestion, in the causation of allergic disorders will form the basis of the present paper. In Table I is recorded a series of ten subjects suffering from allergic disorders caused by the ingestion of foods which contained karaya, tragacanth, or arabic. Direct

*From the Department of Allergy, the Gouverneur Hospital.

†Read before the Fifth Annual Meeting of the American Academy of Allergy, Atlantic City, N. J., Dec. 6-8, 1948.

TABLE I. ALLERGIC DISORDERS IN TEN SUBJECTS RESULTING FROM INGESTION OF FOODS CONTAINING THE VEGETABLE GUMS

CASE AND SUBJECT	AGE AND SEX	GUM-CONTAINING FOODS CAUSING SYMPTOMS	CLINICAL MANIFESTATIONS OF ALLERGY	SKIN-REACTING GUMS	OTHER ALLERGENIC SENSITITIES OF IMPORTANCE
1. K. L.	22 F.	Cream cheese, fillings in candies, marshmallows	Vasomotor rhinitis, gastrointestinal symptoms, epigastric distress, nausea, belching, flatulence	Karaya *Tragacanth** Arabic	Dust
2. L. K.	11 M.	Commercially prepared pie crust mixtures, marshmallows, certain brands wheat cakes, certain brands cream cheese, commercially prepared cake icing, gelatine	Vasomotor rhinitis, gastrointestinal symptoms, urticaria, allergic cough	Arabic Karaya	Dust, animal epithelia, orris root, cotton, silk, chocolate, fish, paprika, mushroom
3. S. S.	35 M.	Commercially prepared white sauce, commercially prepared mustard, processed cheddar cheese, processed Swiss cheese, commercially prepared potato salad	Urticaria, angioedema	Tragacanth Arabic	Dust
4. R. B. A.	37 F.	Commercially prepared cake icing, commercially prepared whipped cream, ice cream mixtures	Vasomotor rhinitis	Karaya Arabic	Dust
5. J. G.	29 M.	Commercially prepared cake icing, fillings in candies, cream cheese	Gastrointestinal allergy: epigastric distress, belching, flatulence, diarrhea	*Karaya* *Tragacanth* Arabic	Dust, animal epithelia, tobacco, timothy, ragweed, mushroom, spinach, banana
6. A. S.	13 M.	Certain brands of tooth paste	Vasomotor rhinitis: clogged nose, running nose, sneezing	*Arabic* Karaya Tragacanth	Dust
7. M. G.	45 M.	Certain brands of gum	Bronchial asthma	Tragacanth Arabic	Trees, grasses, ragweed, dust
8. G. C.	35 M.	Gelatines, commercial whipped cream in "charlotte russe," fillings in candies	Urticaria, angioedema, itchy gums	Karaya Tragacanth Arabic	Dust, trees, ragweed, animal epithelia
9. B. D.	16 F.	Ice cream mixture, frozen custard	Vasomotor rhinitis, bronchial asthma	*Karaya* *Tragacanth* Arabic	Dust, trees, grasses, ragweed, animal epithelia
10. L. D.	55 M.	Commercially prepared salad dressings, shrimp sauce and other	Vasomotor rhinitis: nasal congestion, rhinorrhea, clogged nose, sneezing	Karaya Tragacanth *Arabic*	Trees, grasses, dust, chocolate, orange

SUMMARY OF TABLE I

TOTAL NO. OF SUBJECTS	AGE RANGE	NUMBER OF INDIVIDUAL FOODS CONTAINING THE GUMS	TOTAL NUMBER OF ALLERGIC MANIFESTATIONS	NUMBER GIVING POSITIVE SKIN REACTIONS TO THE GUMS		
				KARAYA	ARABIC	TRAGACANTH
10 7 males 3 females	11-55 years	19	7	8	10	8

Cake icing (commercially prepared)	Bronchial asthma
Cheddar cheese (processed)	Allergic cough
Cream cheese	Angioedema
Fillings in candies	Gastrointestinal allergy
Frozen custard	Urticaria
Gelatins	Vasomotor rhinitis
Gum (certain brands)	Itchy gums
Ice cream mixtures	
Gelatin	
Marshmallows	
Mustard (commercially prepared)	
Pie crust (commercially prepared)	
Potato salad (commercially prepared)	
Salad dressings (commercially prepared)	
Shrimp sauce (commercially prepared)	
Swiss cheese (processed)	
Tooth paste (certain brands)	
Wheat cakes (certain brands)	
Whipped cream (commercially prepared)	
White sauce (commercially prepared)	

skin tests with the gums, clinical trial and elimination, and positive serologic findings confirmed these suspicious allergens as the causative agents. The allergic manifestations recorded in Table I were not only gastrointestinal reactions but consisted also of many other systemic reactions of allergy such as bronchial asthma, generalized urticaria, vasomotor rhinitis, and so forth. Elimination of the suspected irritant foods obviated the symptoms. Clinical trial repeatedly reproduced these symptoms.

SEROLOGIC STUDIES

Four sera in this group were found to contain abundant antibodies, and thus were suitable for passive transfer and neutralization studies. Results of these studies are shown in Tables II and III, respectively. The dominant gum antigens in these four subjects were: tragacanth and karaya in J. G. and B. D., and arabic in A. S. and L. D.

TABLE II. PASSIVE TRANSFER TESTS IN SERIAL DILUTIONS ON THE FOLLOWING GUM-SENSITIVE SERA

	These sites were tested 48 hrs. later with 1/40 to 1/20 c.c. of:		
1. J. G. serum.—Test sites of J. G. serum in serial dilutions were introduced into the back of a nonallergic donor	1% gum karaya	1% gum tragacanth	1% gum arabic
	Reactions		
Concentrate	marked	marked	marked
1 : 10	moderate	moderate	slight to moderate
1 : 100	slight	slight	negative
2. B. D. serum introduced in the same manner			
Concentrate	marked	marked	marked
1 : 10	slight	moderate to marked	slight
1 : 100	negative	slight	negative
3. A. S. serum introduced in the same manner			
Concentrate	moderate	moderate	marked
1 : 10	slight	slight	moderate
1 : 100	negative	negative	negative
4. L. D. serum introduced in the same manner			
Concentrate	moderate	moderate	marked
1 : 10	slight	slight to moderate	marked
1 : 100	slight	slight	slight

As can be seen from Table II, J. G., B. D., L. D., and A. S. showed strongly positive sera which transferred passively on a nonallergic donor.

From the neutralization studies, the following concrete conclusions may be drawn with respect to cross reactions between (1) tragacanth and karaya and (2) between tragacanth and arabic:

(1) In three of the four sera studied the evidence is presented that tragacanth neutralizes karaya. In one serum (J. G.) in which the addition of tragacanth to the serum failed to neutralize karaya, karaya was an unusual highly dominant clinical antigen in this subject.

(2) It was previously shown by the writer[11] that tragacanth neutralizes arabic, but arabic will not neutralize tragacanth. Apparently this pertains only in a serum in which tragacanth is the more dominant antigen. In the present study upon these highly gum-sensitive sera, it is evident that the clinical sensitivity dominance of either of these two antigens determines the capacity of one of them either to neutralize or not to neutralize its mate.

INGESTION OF THE POWDERED GUMS IN SENSITIVE SUBJECTS

Experimental Studies.—The vegetable gums, karaya, tragacanth, and arabic, were compounded in powder form and 300 mg. of the mixed gums were calculated by weight to be contained in each powder
4 subjects (cases 5, 6),

9, and 10) were fed powders containing these gums, and one subject (Case 1) was fed candies containing gum fillings. Results of these experiments were observed and recorded as follows:

Ingestion Experiment: Case 1 (R. L.)* had manifested the clinical forms of allergy as vasomotor rhinitis and gastrointestinal disturbance from the ingestion of foods containing vegetable gums. A brand of cream cheese, fillings in candies, and marshmallows were found to be the incriminating sources of allergic symptoms.

TABLE III. NEUTRALIZATION TESTS

	Three sites of .1 c.c. of each mixture for each serum were introduced into the skin of a nonallergic donor. After 48 hours these sites were tested with 1/40 to 1/20 c.c. of:		
A. Equal parts of J. G. serum and the three gum antigens of 1% concentration were mixed separately in vitro as well as equal parts of J. G. serum and saline as a control:*	1% gum karaya	1% gum tragacanth	1% gum arabic
	Reactions		
J. G. serum + karaya	slight	marked	moderate
J. G. serum + tragacanth	marked	moderate	moderate
J. G. serum + arabic	moderate	marked	slight
B. Same method of mixture, using B. D. serum as follows:			
B. D. serum + karaya	very slight	marked	moderate
B. D. serum + tragacanth	slight	moderate	negative
B. D. serum + arabic	moderate	marked	negative
C. Same method of mixture, using A. S. serum as follows:			
A. S. serum + karaya	very slight	moderate	marked
A. S. serum + tragacanth	negative	very slight	marked
A. S. serum + arabic	slight	slight	slight to negative
D. Same method of mixture, using L. D. serum as follows:			
L. D. serum + karaya	very slight	moderate	moderate to marked
L. D. serum + tragacanth	slight	very slight	marked
L. D. serum + arabic	slight	moderate	slight

*Serum plus saline controls were carried out in each of the four sera; the results resembled closely the serum dilution passive transfer tests given in Table II.

The following ingestion experiment was carried out in this subject: (This patient's favorite candies had been declared, by Dr. Pacini of Universal Colloid Company, to contain the vegetable gums.) For the experiment, Dr. Pacini arranged with a candy manufacturer to make up candies in two distinct layers. The top layer contained the vegetable gums, the bottom layer contained no vegetable gums. The patient, of course, had no knowledge of the contents of the candies. The layers were interchanged and fed to the patient at various intervals. Onset of the gastrointestinal symptoms in the form of belching, nausea, epigastric distress, and flatulence were invariably reported after ingestion of the candies containing vegetable gum fillings.

Ingestion Experiment: Case 5 (J. G.)* had been found markedly sensitive to karaya, tragacanth, and arabic. Trial and error methods disclosed the fact

*See Table I.

that ingestion of certain commercially prepared cake icings, certain fillings in candies, and certain brands of cream cheese known to contain the vegetable gums had invariably resulted in symptoms of epigastric distress, belching, flatulence, and a most disturbing severe diarrhea.

The following experiment was undertaken: The patient was given powders each containing 300 mg. of the mixed gums, namely, karaya, tragacanth, and arabic, to be taken every two hours. Six hours after the initial ingestion of the powders (after an intake of 900 mg.) there resulted a soft stool. After an intake of 1,200 mg. there resulted a definite diarrhea; there were also associated symptoms of epigastric distress, belching and flatulence. A single dose of 2,100 mg. of the powders produced, within three hours, all of the symptoms stated above.

Ingestion Experiment: Case 9 (B. D.)* had been found markedly sensitive to karaya, tragacanth, and arabic. Ingestion of ice cream and frozen custard known to contain the vegetable gums produced symptoms of vasomotor rhinitis and bronchial asthma. Ingestion of the same foods free from the vegetable gums was normally tolerated.

The following experiment was carried out: on the morning of June 5, 1948, the patient was given powders, each containing 300 mg. of mixed gums, namely, karaya, tragacanth, and arabic, every two hours. Soon after the intake of the powders, the patient suffered marked nasal congestion, rhinorrhea, and blocked nasal passages. After the third dose of the powders she sensed definite constriction in her chest, with audible wheezy respirations. Asthmatic breathing was marked at about 3 P.M. That afternoon the patient was forced to leave school and was confined to the house the rest of the day. She continued to take the powders in the late afternoon and, after having taken 1,500 mg., the symptoms of vasomotor rhinitis and bronchial asthma became markedly and progressively intensified. She was forced to resort to ephedrine sulfate capsules for relief of the symptoms.

Ingestion Experiment: Case 6 (A. S.)* had manifested symptoms of severe vasomotor rhinitis when applying certain brands of tooth paste as a dentifrice. The patient was found markedly sensitive by direct skin test and by passive transfer to the vegetable gums, karaya, tragacanth, and arabic.

The following experiment was carried out on this subject: At 5 P.M. of May 10th, the patient was given a total of 2,100 mg. of mixed gums in powder form, with one glass of water. At 5.20 P.M., there occurred marked clogging of the nose, continuous marked rhinorrhea and profuse discharge, and a good deal of sneezing. Examination of the nose revealed bilateral edema of the mucous membranes; the right inferior turbinate appeared greatly engorged and water-logged. There was marked obstruction on both sides of the nose due to the edema, rhinorrhea, and mucous discharge.

The control subject was G. L., 15 years of age. The skin test to karaya was 4 plus and to tragacanth and arabic, respectively, 2 plus and 1 plus. This subject gave a negative history regarding the ingestion of gum-containing foods. He was considered clinically nonsensitive to the gums.

*See Table I.

On the same day and at the same time the ingestion experiment was carried out on patient A. S., the control subject was given a similar amount of the gums (2,100 mg.) in powder form. The result was negative.

Ingestion Experiment: Case 10 (L. D.)* had been clinically sensitive to the following gum-containing foods: salad dressings, shrimp sauce, and other sauces known to contain the vegetable gums. The following events served as proof in this subject:

The direct skin test with karaya was moderate; to tragacanth, it was marked; and to arabic, it was markedly active.

TABLE IV. INGESTION EXPERIMENTS: SUMMARY TABLE

SUBJECT	AGE AND SEX	FEEDING METHOD EMPLOYED IN REPRODUCING SYMPTOMS	OTHER METHODS OF REPRODUCING SYMPTOMS	CLINICAL MANIFESTATIONS OF ALLERGY PRODUCED	TIME INTERVAL OF ONSET; DURATION OF SYMPTOMS
R. L.	22 F.	Vegetable gums in fillings of candies on top layer; no gums on bottom layer; layers interchanged	Feeding cream cheese containing gum, controlled by other types not containing gum, and marshmallows	Epigastric distress, belching, flatulence, nausea	Onset within minutes; lasted many hours
J. G.	29 M.	*May 28-29:* 300 mg. mixed gums in powder form every 2 hrs. Total: 1,200 mg. *June 10:* 2,100 mg. mixed gums in powder form, in one dose	Gum-containing cake icings, certain fillings in candies, certain brands of cream cheese	Epigastric distress, belching, flatulence, diarrhea	Onset within 4 to 6 hrs.; lasted 12 hrs.
A. S.	13 M.	2,100 mg. mixed gums, powder form, in one dose, with one glass of water	Certain brand of tooth paste	Severe acute symptoms of vasomotor rhinitis	Onset in 20 min.; lasted 1 hr.
B. D.	16 F.	300 mg. mixed gums in powder form every 2 hrs. Total: 1,500 mg.	Gum-containing ice cream, frozen custard	Vasomotor rhinitis, bronchial asthma	Onset within 6 hrs.; lasted 24 hrs.
L. D.	55 M.	2,100 mg. mixed gums in powder form with one glass of water	Gum-containing salad dressings, shrimp sauce, etc. Intradermal tests with the gums	Severe acute symptoms of vasomotor rhinitis	Onset within 10 min.; lasted 3 hrs.

In this summary table of the ingestion experiments we note particularly the following: in the cases of R. L., A. S., and L. D., the onset of symptoms occurred within minutes and lasted many hours in the first and last instances, and one hour in the case of A. S.

In the cases of J. G. and B. D., the onset of symptoms occurred within four to six hours, and lasted twelve hours in the former and twenty-four hours in the latter.

*See Table I.

On June 10, 1948, when tested intradermally with these gums for the first time, a reaction manifested itself in the form of severe vasomotor rhinitis, characterized by marked nasal congestion, rhinorrhea, clogging of the nasal passages, and severe sneezing. This reaction lasted three hours and was only slightly ameliorated by Trimeton.

On June 22, 1948, the patient was examined and his general condition was found excellent; the condition of the nose and throat were also excellent. He was again tested intradermally with karaya, tragacanth, and arabic. Reactions to the skin tests were positive, similar to the previous results, and a similar reaction, severe vasomotor rhinitis, resulted. This also lasted three hours.

On July 25, 1948, the patient was given 2,100 mg. of the mixed gums, karaya, tragacanth, and arabic, with a glass of water. A response similar to the above two reactions resulted.

TABLE V. RELATION OF THE DOMINANT ANTIGENS AS DETERMINED BY SEROLOGIC STUDIES AND DIRECT SKIN TESTS TO THE OBSERVED CLINICAL MANIFESTATIONS IN FIVE HIGHLY SENSITIVE SUBJECTS

SUBJECT	DOMINANT ANTIGEN BY DIRECT SKIN TEST	DOMINANT ANTIGEN BY SEROLOGIC RESULTS	CLINICAL MANIFESTATIONS
R. L.	Tragacanth	Not available	Gastrointestinal allergy
J. G.	Tragacanth Karaya	Tragacanth Karaya	Gastrointestinal allergy
A. S.	Arabic	Arabic	Vasomotor rhinitis
L. D.	Arabic	Arabic	Vasomotor rhinitis
B. D.	Karaya Tragacanth	Karaya Tragacanth	Bronchial asthma and vasomotor rhinitis

In four of these subjects, serologic studies were made, in addition to direct skin tests for the determination of the dominant antigen. In the two subjects with gastrointestinal allergy, tragacanth and karaya were the dominant antigens. In the two subjects with vasomotor rhinitis as the allergic manifestation, gum arabic was the dominant antigen; while in the one subject with bronchial asthma and an associated vasomotor rhinitis, karaya and tragacanth were the dominant antigens.

DISCUSSION

The present report deals with allergic disorders caused by the ingestion of the vegetable gums, karaya, tragacanth, and arabic. These gums were contained in certain brands of foods to add bulk, thickness, and binding qualities, for the purpose of "enriching" the foods and making them "heavy."

From the figures supplied by various importing houses[12] regarding the amount, in pounds, of these three gums consumed annually in the United States, it would appear that, if distributed equally per capita (on the basis of the 1940 census figures for the total population of the United States), there is a possibility that each person could be exposed to an average of 300 mg. of the gums, karaya, tragacanth, and arabic per day. Therefore in our ingestion experiments we used 300 mg. of the mixed gums in powdered form, and results obtained showed that very small amounts of these substances were capable of producing severe allergic symptoms. This fact has been previously reported.[10]

It is also possible that on certain days the gum intake from foods for certain individuals might be doubled or even tripled; for it may happen that on a

particular day an individual would include in his ingestion menu a complete five-cent package of gum drops, some gum-established ice cream, a gum-emulsified salad dressing, a gum-containing piping or icing, a gum-rich pie filler, and similar items for which the gums are used. This would naturally result in increased allergic symptoms and would account for gradations of symptomatology from the use of the gums in certain allergic patients.

Thus our patient J. G., who reacted with gastrointestinal symptoms from the gum-containing foods, would on some occasions have suffered only belching, epigastric distress and flatulence, and perhaps only a soft stool, while on other occasions a severe diarrhea would have been an additional symptom.

In a general way the major clinical manifestations of allergy resulting from the ingestion of the vegetable gums encountered in our ten subjects were bronchial asthma, urticaria, angioedema, vasomotor rhinitis, and gastrointestinal symptoms. There was no instance of migraine, acute or chronic eczema, or other manifestations of allergy traceable to the ingestion of the vegetable gums.

The four positive sera made available in the present series lent conclusive proof of the role of the gums as sensitizing allergens in predisposed persons, and served as a means in determining the dominant antigens in these subjects.

The vegetable gums employed otherwise than in foods were the subject of a previous study,[11] indicating their sensitizing qualities by inhalation and external contact. It should be stressed that a considerable amount of gum arabic finds its way into adhesives, and a considerable amount of gum tragacanth is used in cosmetic and pharmaceutical products; also that large quantities of karaya are contained in hair dressing, wave set, and other lotions employed for external application. In view of the wide usage of these gums, it is necessary to include them constantly in our testing tray.

Gums chemically similar to these principal commodities are frequently employed in their stead, such as cherry tree gum as a substitute for arabic, locust as a substitute for karaya, and algin and other gums as partial substitutes for tragacanth. Similarly, to counteract untoward reactions frequently occurring from laxatives, colloid laxatives are substituted at present. Synthetic mucilages such as polyvinyl alcohol and methyl cellulose are now employed to provide hydrophilic colloids which do not produce the usual side reactions of the vegetable gums. These hydrophilic colloids are not absorbed by the intestinal mucosa, are not degraded by intestinal enzymes, and are not antigenic.[13]

The following data regarding the use of the gums in the foods consumed by our patients has been supplied to the author and, by permission, are herewith listed:[14]

Gravies.—A frequent practice is that of adding gum tragacanth to impart thickening ordinarily achieved with flour and starches.

White Sauce.—As in the case of gravies, gum thickeners are used, principally tragacanth.

Prepared Mustard.—Some brands are thickened with karaya or locust to prevent separation.

Processed Cheddar Cheese.—Most processed cheeses contain gums, particularly karaya, locust, and, very rarely, tragacanth.

Processed Swiss Cheese.—See *Processed Cheddar Cheese.*

Prepared Potato Salad.—The dressing is thickened with karaya.

Cake Icing Mixtures.—These contain gum arabic and, less often, either karaya or locust.

Commercial Whipped Cream.—Frequently stabilized by a mixture of sugar, cornstarch, gelatin, agar-agar, salt, and vanilla. During the war, difficulty in procurement of agar led to its replacement in many instances by either karaya, locust, tragacanth, or, most generally, sodium alginate. This practice still persists.

Ice Cream Mixtures.—These contain karaya and/or locust. Sodium alginate is used in some, but karaya is more prevalent.

Fillings in Candies.—Soft centered candies usually contain considerable gum arabic. In some instances karaya is used.

Cream Cheese.—Packaged cream cheese is labeled with an indication that it carries a "vegetable stabilizer," most always karaya.

Tooth Paste.—Some tooth paste is free from gums, but some carry uronic acid gum, generally karaya and occasionally locust as a substitute for karaya.

Certain Brands of Chewing Gum.—The scarcity of chicle during the war resulted in the development of numerous gum substitutes, and in the change of several chewing gum formulas which included then (as many still include), gums such as tragacanth and, more rarely, karaya.

Charlotte Russe.—The whipped cream filling is stiffened with a gum, which replaces the agar-agar formerly used. Karaya is the commonest choice.

Shrimp Sauce.—See *White Sauce.*

Salad Dressing.—With the exception of mayonnaise, which would be considered adulterated by the Government if it contained gums, other forms of salad dressing substitutes for mayonnaise contain thickening agents, principally karaya, locust and, less frequently, tragacanth.

Marshmallows.—Ordinarily marshmallows are gelatine confections and are devoid of added gums. Occasionally, however, gum acacia is added. When marshmallows are prepared from *Althea officinalis*, it is a trade secret to employ gum acacia to insure the rigidity of the confection.

Certain Brands of Wheat Cakes.—Few prepared griddle cake flours disclose the presence of gum in the list of ingredients. Despite this, a number of prepared flours contain small amounts of the order of one-half per cent of karaya.

SUMMARY

1. The vegetable gums, tragacanth, karaya, and arabic, may cause allergic disorders by ingestion in sensitive subjects.

2. Ingestion experiments with these gums carried out in five sensitive subjects established the etiological relationship of these allergens to the allergic manifestations produced.

3. Passive transfer tests in serial dilutions as well as neutralization studies confirmed the allergenic nature of these gums in the sensitive subjects studied.

4. Widespread employment of these gums in food and other industries is a hazard for the sensitive individual from ingestion, inhalation, and surface contact of these gums.

5. Substitution of other gums for tragacanth, karaya, and arabic, and of synthetic mucilages and celluloses will help in offsetting the danger of sensitization encountered from their use.

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DISCUSSION

STEARNS S. BULLEN, SR., Rochester, N. Y.—Allergic reactions following exposure to vegetable gums are not very frequent, but when they do occur they are likely to produce severe and explosive symptoms.

It is known that the vegetable gums produce symptoms in susceptible people by contact and by inhalation. Dr. Gelfand has given further evidence that the ingestion of these gums may result in allergic reactions.

His case reports illustrate very well the fact that an ingested substance may produce reactions not only in the gastrointestinal tract but also on the skin and the mucous membrane of the respiratory tract and further that reactions may occur in the respiratory tract without any symptoms referable to the gastrointestinal tract. Also in one of his cases, although there was a four plus reaction on skin testing, no symptoms occurred on exposure to the gum. While all these phenomena are well known, it is seldom that they are so well illustrated in a small group of cases.

The constitutional reaction occurring on skin testing in one case which was intentionally reproduced should serve as a warning that skin testing with these vegetable gums is not without danger and should be done with the same care as with extracts of fish, cottonseed, and so forth.

Dr. Gelfand's list of substances in which these vegetable gums are used on a commercial scale will be of great aid to the clinician.

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Gum Tragacanth in Iran

Several species of the genus Astragalus, growing wild in Iran, Iraq, Turkey, Afghanistan and adjacent Russia are the commercial sources of this exudate, obtained by tapping the branches or roots. Its hydrophilic and colloidal properties are of value in the manufacture of ice cream, liquors, lotions, sizings and other industrial products.

HOWARD SCOTT GENTRY *

Introduction

In Iran gum tragacanth plants have never been cultivated in any way, only exploited in the wild according to the circumstances of market and the country inhabitants. Until recently they were given no particular attention as a continuing asset, but a national law or regulation now prohibits destruction of any gum tragacanth plant. However, at least some of the species are still used by the tribal people for badly needed firewood, and extensive stands of the larger highland shrubs are reported to have been destroyed in recent times for this purpose. Uses of the gums have been increasing in recent years, and higher prices have failed to increase supply. The growth of the industry appears, therefore, to depend upon conservation of existing stands and their increase. Also, the question of cultivation appears to be at hand.

The area of gum tragacanth in Iran has an arid temperate climate of the Mediterranean type, or with winter-spring precipitation and rainless summers. Annual average precipitation ranges from about five to 20 inches, part of which falls as snow, increasingly so in the higher elevations. Frost is of annual occurrence through the area. It is light and of low intensity in southern regions, as about Shiraz and Bam. The frosts are heavy and enduring in the high mountain elevations and may reach as low as 0° F. The growing season of the southern regions and lower elevations begins with the first fall or winter rains and continues according to temperature and moisture until early summer, when soil moisture is virtually exhausted. In higher elevations and northward the growing season is much shorter, following snow melt and continuing until midsummer.

In Iran the plants occupy arid and semi-arid highland slopes between 4,000- and 10,000-feet elevations. They are partial to the limy well-drained slopes and footplains of the mountains, frequently appearing most at home on rocky soils, all of which belong to the category of gray earths. The better producers of gum tragacanth appear to be the small, desert, cushion-type bushlets, only a few inches tall and low-spreading, in the lower elevations between 4,000 and 7,000 feet. The larger species, forming thick-branched shrubs, three to six feet tall, are generally restricted to elevations above 7,500 feet and produce lower grades of gum. Doubtless, there are exceptions to this, but such is the general case for the area visited by the author in 1955, namely, the Zagros Mountains from Shiraz to Tabriz, and the adjacent

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slopes of the El Borj Range. The dry summer climate is important to the production of gum, and rains are reported to curtail harvest.

The Plants

Gum tragacanth plants are perennial legumes, all belonging to the genus *Astragalus*, section Tragacantha, characterized by spine-tipped leaf rachises; sessile or subsessile flowers, glomerate in the axils of the leaves; and one-seeded pods enclosed in hairy persistent calyces. The inflorescence is conspicuously white hairy, in some species giving the effect of a cottony ball investing the distal part of the branch (Fig. 7). From this appearance come such native names as "panbeh" and "pachmach", meaning "cotton" or "cotton-like". The midribs of the leaves form a spiny protective armature for the plants, both during the short term they bear leaflets and for many years thereafter. As they lose their green color and turn yellowish to brownish, they apparently perform but little physiological function after the first season of growth. The stipules are enlarged, forming wings at the base of the petiole, clasping the stem and flowers.

The better gum plants are small, low, bushy perennials, frequently of cushion-like form (Figs. 1, 8, 9). They seldom reach 30 cm. in height and frequently are only eight to ten cm. tall. They have relatively large tap roots, and it is these which are tapped for gum (Fig. 3). A few of the species form erect bushes or shrubs, the branches of which are tapped for gum, but all of those observed by the author in Persia yield inferior grades of gum. The largest are reported to reach two meters in height. Plants one and one-half meters in height, with thick wide-spreading branches, were observed in the Zagros Mountains.

Although the above-listed characters distinguish gum tragacanth plants botanically, their most remarkable feature is a central gum cylinder. This appears most highly developed in the roots of the smaller species, e.g., *Astragalus gossypinus* and *A. echidnaeformis*, the mature tops of which can be covered by an ordinary hat (Fig. 8). A longitudinal section of a mature root discloses the central cylinder to be completely filled with an opaque gel-like substance without apparent cell structure or pith. The anatomy of tragacanth stem was examined as early as 1857 by von Mohl (2) who found that the pith and medullary rays of the central cylinder metamorphose into the undifferentiated material composing the central uniform mass of gum. Presumably there is a similar ontogeny in the root. The gum cylinder is contained by the woody cylinder with annular growth and with numerous conspicuous medullary rays radiating out from the central cylinder. These also are turgid with gum during the dry summer season. The diameter of the gum cylinder varies a great deal among species and to a less extent between individuals within a species. In non-commercial Tragacantha it is usually only one or, at most, two millimeters in diameter, while in the smaller productive desert plants it is relatively very large, the diameter of the gum cylinder near the root apex measuring as much as eight to ten millimeters or nearly half the total diameter of the root. These plants are true xerophytes with the enlarged gum cylinders appearing as nutrient and moisture storage organs, as is common in many forms of other xerophytes in all arid climates with long dry seasons.

A good gum-producing plant can be determined by cutting the tap root and inspecting the gum cylinder, a procedure which should be useful in selective breeding or seed collection. In some species the gum cylinder is smooth and clean-sided, as in *Astragalus gossypinus;* in others it contains ragged fibers which

may be forced out with the gum, making it difficult to pull off the dried exudate.

The gum is contained in the cylinder at high pressure, as is easily demonstrated during summer by cutting a section of root or stem. The gum will exude from both cut ends, but mainly from the upper. The writer has observed the exudate to reach about an inch in length in a half hour or so. This exudation was also observed and reported by Hanbury (2). The osmotic pressure of this secretion must be very high and may account for some of the coloring and impurities in the exudates, as its contact with cut tissues during extrusion indicates imbibition of cell sap from phloem, cambium or bark. The large gum cylinder of Tragacantha explains why the small plants are capable of yielding amounts of gum quite over-proportionate to their size when compared to other gum producers. With few exceptions, as in *Sterculia urens*, which contains karaya gum in the central pith as well as in other tissues, gum exudates generally originate from bark and phloem.

Generally there are two types of gum produced by two groups of plants: the small gray bushes, "panbeh" or "ghavan panbeh"; and the yellow bushes, "ghavan zardeh" or "ghineh zard". The gray bushes yield the sweeter, clearer, better grades of gum, "maftuli", while the yellow bushes yield the yellowish bitter grades, "kharmoni". The yellow appearance of the latter plants is given by the russet or yellow color of the broad, stem-clasping, persistent stipules. The gray plants are found through the lower elevations, between 4,000 and 7,000 feet, and are sparsely scattered xerophytes, while the yellow bushes may become abundant through the higher elevations between 6,500 and 10,500 feet (Fig. 2). Some of the latter species may reach two meters in stature and are truly shrubs. Demarcation between these two groups is not sharp, for some of the gray plants show yellow stipules, and these or others may yield gums of both ribbon and flake grades. Likewise, some of the yellow species were found to produce ribbon grades 4 and 5. On the whole, however, the distinction is valid and useful.

Nine of the following species are here first reported to yield gum tragacanth, making a total of 23 species now known to produce the gum. Apparently many other species are still to be identified as gum plants, since the Section Tragacantha in Boissier's flora (1) includes 156 species, while that of Parsa (5) lists 104 species, many of which are additions to what was known in Boissier's time. They constitute a very rich aggregate for experimental investigations.

Acknowledgment of the generous assistance of Prof. A. Parsa of Tehran is to be made for his identification of most of the species here listed.

Astragalus adscendens Boiss. & Haussk. "manna"

Spreading shrub, 1–1.5 m. tall, with rather thick, numerous, widespreading, decumbent to ascending stems, forming a flat-topped crown, bearing grayish-green foliage on short spur-branchlets; leaflets 12–14, 6–8 mm. long, narrow, involute, bluntly mucronate; flowers lavender, many-glomerate in the axils and enclosed in the acute hyaline stipules; latter August and September (Fig. 4).

Calcareous rocky slopes of the central Zagros Range from Arak Province

Fig. 1 (Upper). The cushion form of vegetation in the Zagros highlands west of Abadeh, Semiron Province. Among the cushion plants are *Astragalus sengancusis*. The shrub in background is *A. myriacanthus*.

Fig. 2 (Lower). At 9,500 feet elevation in Azerbaijan with Kuhe Sahand in background. The car is surrounded by a yellow bush type of gum tragacanth, *Astragalus* sp.

northwest through Luristan and about Kermanshah, from 8,500 to 10,000 feet elevations. Gentry *s.n.*, ca. 10 km. south of Khonsur, Arak Province.

Gum type-Flake grades obtained by incising the branches. This was one of the first tragacanth species reported botanically by Haussknech in the latter part of the last century.

It also produces a Persian manna, finding a ready market in Isfahan and other towns, where it is made into candy or confection, some of which appears to be exported. The product is produced as a sugar exudate from the leaves during the fall when humidity conditions are favorable. At that season the gatherers, equipped with a stick and light white cloth, go out among the plants. Holding the cloth under the exuding branches, they strike the branches with the stick, and the dry finely granular sugar falls upon the cloth. This sugar exudate from the leaves is not to be confused with the gum, which was not regarded by the author's informants as edible. The sugar exudate is not a result of insect punctures as has been reported for *Tamarix mannifera*.

Astragalus brachycentrus Fisch.
"ghiaeh zard"

Low spreading bush with yellow branches and crown looser than cushion type, 25–40 cm. tall; leaflets small, 4–5 mm., numerous, 10–14, pale green on pale green rhachises which turn yellow in age; flowers small, rather long-deployed along the new growth; June. The species is variable with what appear to be ecotypes; the variety *koïeanus* from Luristan was named by Sirjaev (51). Gum cylinder generally large but ragged with medullary tissue.

Valley and mountain slopes of the Zagros Range from Fars to Hamadan and Luristan, 6,000 to 8,000 feet elevation, where it frequents the rocky calcareous soils. Gentry Nos *14969, 15165*, Bavanat Valley and adjacent mountain slopes, Fars Province, 7,000–8,500 feet elevations; No. *14994*, 32 km. southwest of Abadeh, Fars Province, 8,500 feet elevation; also doubtfully referred here is sterile No. *14968*, Bavanat Valley, Fars Province, 7,000–7,500 feet.

Gum type—Produces the lower grades of ribbon and better grades of flake, and is an important producer in many localities, for instance, Bavanat Valley. The larger bushes are generally cut along the base of the branches or high up on the tap root; the old split scars were frequently observed in the field. Gum samples are reported in Table III.

Astragalus cerasocrenus Bunge
"gonmer"

Forms medium-sized to rather large cushion type bushes, 20–25 cm. tall and 30–50 cm. in crown diameter, with glaucous-green foliage; the 6–8 leaflets 15–20 mm. long and rather remote on a strong rhachis; flower heads elongate, large, and well below the branch tips. Roots 50–70 cm. long with gum cylinder 3–5 mm. in diameter. Varietal differences are notable in the species, and very large specimens were reported to reach as much as one meter tall and two meters broad on the moister more fertile soils of the highlands around 8,000 feet elevation (Figs. 3, 5).

Valley slopes of the Zagros Range from Fars Province to Azerbaijan; reported by Parsa (5) also from Khorasan Province to eastern Iran. Observed on the atrital alluvial and rocky slopes of

Fig. 3 (Upper). *Astragalus cerasocrenus* with the large tap root exposed. It was topped by plowing the previous year. The root was tapped three days prior to photograph and produced the white exudate.

Fig. 4 (Lower). *Astragalus adscendens* in the Zagros Mountains near Khonsur. Besides gum it produces the sugar manna of Persia.

Astragalus globiflorus Boiss.

"golbanbeh"

Low, gray, canescent bushlet with whitish foliage, 3–4 pairs of leaflets, 12–14 mm. long; flowering heads below the new shoots of the season: therefore, flowering early. Except for the light gray foliage, appears very similar to *A. remsocrenus* and *A. senganensis*. Gum cylinder not examined.

Given as southwest Iran by Parsa (5). Gentry No. *14995*, 32 km. southwest of Abadeh, Fars Province, 8,500 feet elevation, on limestone conglomerate.

Gum type—Stated by tappers to be of good quality; no samples obtained.

Astragalus gossypinus Fisch.

"panbeh", "golpanbeh", "ghavan panbeh"

This small gray bushlet has several varieties. On the dry slopes in the Isfahan district it is only 5–8 cm. tall and 15–20 cm. in crown diameter, with gray, canescent foliage; 8–12 ovate pungent leaflets, 3–5 mm. long, along the spine-pointed rhachis. The small pink flowers appear in July and are extended shortly from the cottony white, sessile calyces in wooly white heads about the ends of the short branches. The tap root is small, 1–2 cm. in diameter, 20–30 cm. long, but the gum cylinder, 5–8 mm. in diameter, is relatively very large. In the Kermanshah area, where rainfall is more, the species forms larger plants (Fig. 8).

Along the eastern slopes of the Zagros Mountains from Fars Province to Kermanshah and adjacent northeastern Iraq, where it frequents the dry, calcareous and gypsiferous, well-drained, gray earth soils between 4,000 and 7,000 feet elevations. Widely scattered and never observed in close colonies. Gentry Nos. *15268*, *15269*, ca. 10 km. north of Kermanshah, Ostan 5; *15680*, ca. 35 km. east of Tehran; *15260*, Kuhe Safed near Isfahan; *s.n.*, Estavant, Fars Province.

Gum type—Produces the best or ribbon grades. One of the most important commercial species in the Isfahan and Tehran areas. It is probable that gum from this species also reaches Bagdad via the nomads from the western slopes of the Zagros.

Astragalus microcephalus Willd.

"panbeh", "ghuchu", "golbanbeh", "golpachmach"

Small, low, spreading, gray-canescent bushlets, 8–12 cm. tall, 15–25 cm. broad; 8–12 leaflets, 4–6 mm. long, ovate, pungently tipped; flowers very late, not seen. Gum cylinder well developed, 6–8 mm. in diameter below the root crown. Apparently a variable species (Fig. 9).

Eastern valley slopes along the Zagros Range from Fars Province to the southern slopes of El Borj Range, fid. Parsa (5). Calcareous atrital slopes 4,000–5,500 feet. Gentry Nos. *15078*, Bajga, 15 km. east of Shiraz, Fars; *15164*, Kuhe Bavanat, Fars Province, 8,000–8,500 feet elevation.

Gum type—Ribbon grades and stated by the tappers and merchants of Shiraz to be the best produced in that region. Good clear samples were collected and weighed, Table III.

Astragalus myriacanthus Boiss.

"ghineh cheraghee"

Erect spreading shrubs, 6–14 dm. tall, with thick stems, 5–8 cm. in diameter, thick spreading gray branches, and numerous short spur branchlets with yellow scarious appressed stipules, yellow spreading thorns; the new growth white

Fig. 7 (Upper). *Astragalus elymaiticus* near Shiraz, Fars Province, with root and exudate exposed after digging. Note the ball-like cottony inflorescence at ends of the branches.

Fig. 8 (Lower). *Astragalus gossypinus* near Isfahan with spiraling exudate. This is a mature plant several years old.

pubescent with numerous small flowers in June; leaflets numerous, 16–20, and small, 3–4 mm., ovate, mucronate, gray pubescent. Colonies of these flat-topped bushes are characteristic of the highlands in many localities in the Province of Fars (Fig. 1). The branches of this and related species allegedly burn well and are used for fuel.

Boissier (1) reported this shrub from Dená in the southern mountains. It was observed frequently on excursions through Fars to Charmahal in the central Zagros at elevations between 7,500 and 10,500 feet. Gentry No. *15047*, mountain above Bajga Valley, east of Shiraz, Fars Province, on limestone.

Gum type—Produces low quality gum of flake grades from incisions made along the branches. The amounts obtained are greater than from the roots of the small gray species, but, since the value of the gum is very low, the bushes are untapped in many localities. Gum samples are reported in Table III.

Astragalus senganensis Bunge
" gommer "

Low, dense, erinaceous perennials with pale green foliage; 6–10 leaflets, 15–20 mm. long; short globose flower heads at the ends of the branches. It closely resembles *A. cerasocrenus* from which it is distinguished by the short, distal, globose flower heads (compared to non-distal, oblong flower heads) and by the stamens being attached near the base of the staminal tube (vs. attached high on staminal tube). The tappers encountered did not distinguish between the two species. The gum cylinder of the root is rather small and ragged (Fig. 1).

Mountain and valley slopes of the limestone mountains of the Zagros Range from Fars Province to " montagnes entre Teheran et Ispahan ", Parsa (5). Gentry Nos. *14970*, Bavanat Valley, Fars Province; *14993*, 32 km. southwest of Abadeh, Fars Province.

Gum type—Flake grades, and a rather low producer, Table III.

In addition to the above-listed species, observed and collected in the field as producers of gum tragacanth, the following have been reported by other authors (2, 7, 3) as yielding gum tragacanth.

A. brachycalyx Fisch., from the Anatolian slopes, including southern Kurdistan and northern Iraq. An erect shrub of the yellow group, doubtless cut along the branches and yielding flake grades, as is common in that group.

A. creticus Lam., reported as from Crete and southern Greece.

A. cylleneus Boiss. & Heldr., from the Peloponnese Islands.

A. eriostylus, Boiss. & Haussk., described as a low shrub, 1 meter tall and somewhat erinaceous, from southern Iran.

A. gummifer Labill., from the Anatolian Plateau in Turkey and Armenia as a low spiny bushlet producing much of the gum in earlier times from that area.

A. heratensis Bunge, growing in the region around Herat in Afghanistan and adjacent Khorasan Province of Iran as a low cushion type of plant.

A. kuydicus Boiss., reported as from Chormuh mountain near Bushir, southern Iran.

A. leiocladus Boiss., reported from the Zagros mountains of Arak Province with decumbent branches. It is placed in the section Brachycalyx along with *A. adscendens* and *A. myriacanthus;* so it is presumably tapped along the branches and yields low grades of gum.

A. pycnocladus Boiss., Arak Province in the central Zagros. Forms a low cushion type of plant, closely related to *A. microcephalus*, and therefore is presumably tapped on the root.

A. strobiliferus Royle, described as from the Harirud valley of northwestern Afghanistan and closely related to *A. globiflorus*.

A. stromatodes Bunge, an erinaceous shrublet from the Anatolian region.

A. rerus Oliv., described as from western Iran between Kermanshah and Hamadan, with 8–10 leaflets on a very short rhachis (2 cm.).

It is to be expected that several others of Tragacantha also produce the gum tragacanth of commerce. Very little botanical field work, other than collecting specimens, has ever been done with this group of plants. They are spiny and difficult to handle and to press. It is only to be expected that botanists have frequently passed them by for other easier subjects. In studying the roots in the field, the writer found that several other spiny species, but with pedunculate inflorescences, also produce gum. Samples of gum procured from some of these were pronounced by gum merchants not to be gum tragacanth, but of inferior sorts, and were known in the trade by other names, as "shadeh", "ghooreh" and "oshagh" (Fig. 11). The spiny plants having pedunculate inflorescences are therefore to be excluded from the gum tragacanth group.

Gum Tragacanth in Commerce

History. It is unknown how and when man first discovered gum tragacanth, as its use predates ancient history. Speculatively, he probably used it first as food when impelled by hunger, since ants, goats, sheep and wild sheep appear to be fond of the sweeter kinds. Or he used the more bitter varieties as medicine, for sickness was an adversity that drove companions of the ill to innovation through cult and mysticism. Flückiger and Hanbury (2) were of the opinion that the gum was first used in historical times as an item collected from the bark and that only later, as demand increased, were incisions made to increase supply. It was known to the Greek physicians from the 7th to the 4th centuries B.C. Theophrastus in the 3rd century B.C. wrote that the plants producing it were native to the Peloponnesian Islands and to Crete.

FIG. 9. *Astragalus microcephalus* at Bajga, Fars Province, showing several inches of gum tragacanth from an incision made three days previous.

By 1300 A.D. it was an article of com-

merce to Europe via the trading cities of Italy. Among early travelers from Europe to the Near East, who actually saw and reported the plants with their exudates, are Pierre Belon in 1550 and Tournefort in 1700 (6). The German botanist, Haussknecht of Weimer, visited northwestern Persia in the latter part of the 19th century, and some of Boissier's species are based on his collections. It is one of the oldest drugs in Materia Medica and has been offered in every edition of the U. S. Pharmacopoeia since 1820. A common Persian term for gum is "katira". Some of the gum tragacanth bushes in Fars, home of the Archaemenian kings, are still called "cummer" or "gommer". This is suggestive as the origin of our term "gum", being traceable through French "gomme", Latin as "gummi" or "cummi", and Greek as "kommi". It is about as clear as good gum itself.

Identity. Gums are defined as water-loving, or hydrophilic, colloids occurring as exudates of certain perennial plants. They are truly amorphous without melting point, freezing point, or boiling point characteristics. They are largely carbohydrates containing calcium, magnesium and potassium. Nitrogen, although it may be present, is not an essential constituent, its usual absence distinguishing them fundamentally from proteins which always contain nitrogen. Gums differ from resins in being colloidally soluble or dispersible in water but insoluble in organic solvents and drying oils; resins are insolvent in water but are soluble in drying oils and organic solvents. Gums are quite unrelated to resins in their uses as well as physically and chemically.

The great capacity of gums to absorb water, forming viscous solutions and colloidal gels, has found wide application in industry. Such colloidal solutions are known as "sols". They have low surface tensions and act as protective colloids and stabilizing agents. Tragacanth gum consists of a soluble portion, tragacanthin, and an insoluble portion, bassorin, the latter constituting 60 to 70% of the total. Tragacanthin consists of a ring containing three molecules of glucuronic acid and one molecule of arabinose with a side chain of two molecules of arabinose. The soluble portion gives a colloidal hydrosol solution with water, while the insoluble part swells into a gel. For further chemical exposition the reader had better turn to Mantell (4). Vegetatively the tragacanth gums are unique, as they are the only commercial ones originating solely in a central gum cylinder. In commerce they rank with gum arabica in quantity and quality.

The uses of gum tragacanth are many. The sweeter, clearer, finer sorts, ribbon or "maftuli", are employed in pharmaceutical mixtures; in liquors, to which they add smoothness or "body"; in cosmetics, as the glycine toilet creams; jellies, lotions, dental creams and many others. Confections consume much of the fine grade tragacanth, however, and the American consumer, without knowing, may take it in ice cream, candies, syrups, jellies, salad dressings, mayonnaise, pickles, sauces, chutneys, flavour emulsions and egg substitute preparations. The bitter, less clear tragacanth gums, flake or "kharmony", are employed in dyes, paper sizings, waterproofing for fabrics, as a creaming agent for rubber latex, and so on. They have an extensive use in the textile industry as constituents for the sizing of yarns and threads, for the stiffening of felt goods, for leather dressings, and for transparent finishing of silk or rayon. There is also large application in calico printing, and much of the local consumption in Iran is by that industry. Altogether it is a multiple purpose resource for food and textile products.

Imports. United States imports of gum tragacanth during the past two decades have averaged over two millions

pounds annually at an annual cost of over one and one-half million dollars. Had there not been a decline in supply, imports would have been more during the past decade. Source of imports from 1945 through 1954 is shown in Table I. Iran is our principal supplier, followed by Iraq and Turkey. Imports from India, the United Kingdom and other subsequent and continuing decrease. This appears due to limitation of the existing resource, as was pointed out by Iranian merchants. In view of the increasing use or demand and the general deterioration of the wild stands of *Astragalus* shrubs, it is very unlikely that price will lower, unless more satisfactory substitutes are found.

TABLE I. UNITED STATES IMPORTS OF GUM TRAGACANTH DURING THE LAST DECADE. NOT SHOWN AS TO SOURCE BUT INCLUDED UNDER "OTHERS" ARE TWO SUBSTANTIAL LOTS FROM LEBANON AND ONE LOT FROM SAUDI ARABIA. SOURCE, CENSUS BUREAU, UNITED STATES DEPARTMENT OF COMMERCE

Year	Iran		Iraq		Turkey	
	lbs.	*U.S.$*	*lbs.*	*U.S.$*	*lbs.*	*U.S.$*
1945	2,600,997	2,027,976	8,907	27,934	406,477	194,306
1946	3,667,393	3,305,095	42,629	56,317	4,389	7,981
1947	3,017,240	3,153,777	27,914	95,310	3,901	1,663
1948	2,600,809	2,343,079	220	308	.....	.....
1949	1,181,972	1,074,895	.....	.....	3,103	1,126
1950	2,817,455	1,889,991	2,580	4,405	1,587	980
1951	1,251,401	1,455,353	.....	.....	627	998
1952	2,391,652	1,921,714	.....	.....	.....	.....
1953	1,335,336	719,010	.....	.....	.....	.....
1954	1,018,160	639,611	151	319	.....	.....
Totals	21,982,715	17,920,830	82,434	184,623	420,084	207,054

Year	India		United Kingdom		Europe and others	
	lbs	U.S.$	lbs	U.S.$	lbs.	U.S.$
1945	.....	.....	50	44	.....	.....
1946	123,170	73,902	.....	.....	.....	.....
1947	13,387	11,157	.....	.....	150	630
1948	.....	.....	3,330	5,808	.....	.....
1949	.....	.....	17,509	6,811	39,600	13,199
1950	2,325	950	48,153	41,919	25,822	23,212
1951	.....	.....	6,815	2,211	9,788	19,866
1952	673	221	20,851	9,323	5,491	4,958
1953	6,335	7,825	2,809	5,305	.....	.....
1954	.....	.....	.....	.....	.....	.....
Totals	145,800	94,358	99,508	72,051	80,851	62,195

European countries are trade detour lots which originate in Near East countries.

Figure 10 indicates the average price and quantity imported from 1931 through 1954. It is apparent that supply and price have not been closely correlative from the war years to the present, as a great increase in price was followed by only a limited increase in supply with

United States imports represent only a part of the Iranian production. Table II, prepared by American Consul John Ordway, in Isfahan, is based on Iranian customs statistics and shows total Iranian exports for nearly three years. This constitutes the bulk of Iranian production, as it is reported that relatively little is consumed locally. During this

TABLE II. IRANIAN EXPORTS OF GUM TRAGACANTH FROM MARCH, 1948, TO JANUARY, 1951 (IRANIAN CALENDAR 1327–29). SOURCE, AMERICAN CONSULAR REPORT, JOHN ORDWAY, 1951

	Country	Metric tons	Value in Rials*
March 1948 to March 1949	United Kingdom	760	35,309,000
	United States	768	32,430,000
	France	285	11,270,000
	Germany	259	8,640,000
	India	153	8,204,000
	Other countries	292	9,382,000
	1948–1949 Total	2,497	105,235,000
March 1949 to March 1950	United Kingdom	795	41,688,000
	United States	163	6,151,000
	France	546	9,710,000
	Germany	255	9,237,000
	India	106	3,981,000
	Other countries	277	13,191,000
	1949–1950 Total	2,142	83,961,000
March 1950 to Jan. 1951	United Kingdom	995	57,982,000
	United States	584	28,032,000
	France	622	20,772,000
	Germany	243	10,744,000
	India	155	6,107,000
	Netherlands	356	14,904,000
	Other countries	333	16,795,000
	1950 (10 months) Total	3,288	155,336,000

* The official exchange rate is Rials 32 equals $1.00 but the rate of Rials 48 to $1.00 is generally used in export transactions.

term these exports amounted to nearly seven million dollars in value, representing hard currency for Iranian commerce. The United Kingdom and the United States took about half of total Iranian exports during this period, with about 20% going to the United States. London has long been a principal center of world trade in gums.

The Industry in Iran. While in Iran the writer interviewed gum merchants in Shiraz and Isfahan, and a number of tappers and contractors elsewhere. The following is a digest of the information obtained regarding gum commerce in that country.

The commercial grades of gum tragacanth have been more or less standardized. In recent years in Iran a company has been formed to regulate exports and to preserve uniform grading and price. Tehran, Isfahan and Shiraz have been designated as the grading and exporting centers, and all gum produced must pass through the exporters in those towns. Grading is done by experts or trained youths on the basis of color and quality as it appears in the varied lots received from the collectors, with but incidental attention to specific plant origin. Certainly the commercial graders have no concept of species nor of the properties to be correlated with such. Samples collected were taken for commercial grading to one of the principal exporters of Isfahan. Single samples, consisting of the exudate from one cut on a single plant in several cases, were broken and separated into two or even three grades solely on the basis of color differences. However, some of the samples from related *Astragalus* shrubs were immediately identified as not being gum tragacanth, showing the perceptive acumen of an experienced merchant. The commercial grades with current prices per kilogram Isfahan, as of July, 1955, were:

Ribbon or "naftuli"		
No. 1	540 rials	$7.20
2	430	5.70
3	330	4.40
4	250	3.33
5	160	2.13
Flake or "kharmoni"		
No. 1 (26)	140 rials	$1.87
2 (27)	120	1.60
3 (28)	75	1.00
4 (55)	55	.73
5 (31)	45	.60

No. 1 ribbon in Shiraz was quoted at 500 rials, where 550 was reported as the Tehran price for the same grade, the difference being attributed mainly to transportation costs. The same grade was reported to have sold the previous year for around 440 rials. According to these prices, it would appear that the U. S. consumer pays $5–8 per kg. for top grade

gum tragacanth, which he consumes in ice cream, liquors, confections and other items.

Import statistics give no indication of such price ranges nor of the amounts produced of such grades. Shiraz reported that only about one percent of production in that area is of top grade, while the flake or inferior grades constituted the bulk of production. The Isfahan area produces a higher proportion of ribbon grades than does Shiraz, but flake grades are reported as constituting most of the production in that area and elsewhere in Iran. No figures are available showing the respective quantities of ribbon and flake grades produced [1].

These quotations are said to be 20 to 30% higher than in 1954. Merchants justified the prices of 1955 by the poor crop that year, which was estimated to have been only about half that of the preceding year. The light production was attributed to: (*a*) heavy floods of the previous winter which allegedly washed out many plants; (*b*) a general lack of spring rains; (*c*) scarcity of tappers who were attracted to more lucrative labor; (*d*) steady decline in the number of plants during the last 40 years as a result of burning the shrubs for fuel and of attempts to control the bug pests of *Agrostis* and *Aelia*, which over-winter in the plants. It is reported that 40,000 to 50,000 people are employed seasonally in this industry, the great majority being the village people who cut and collect the gum. One village in the prov-

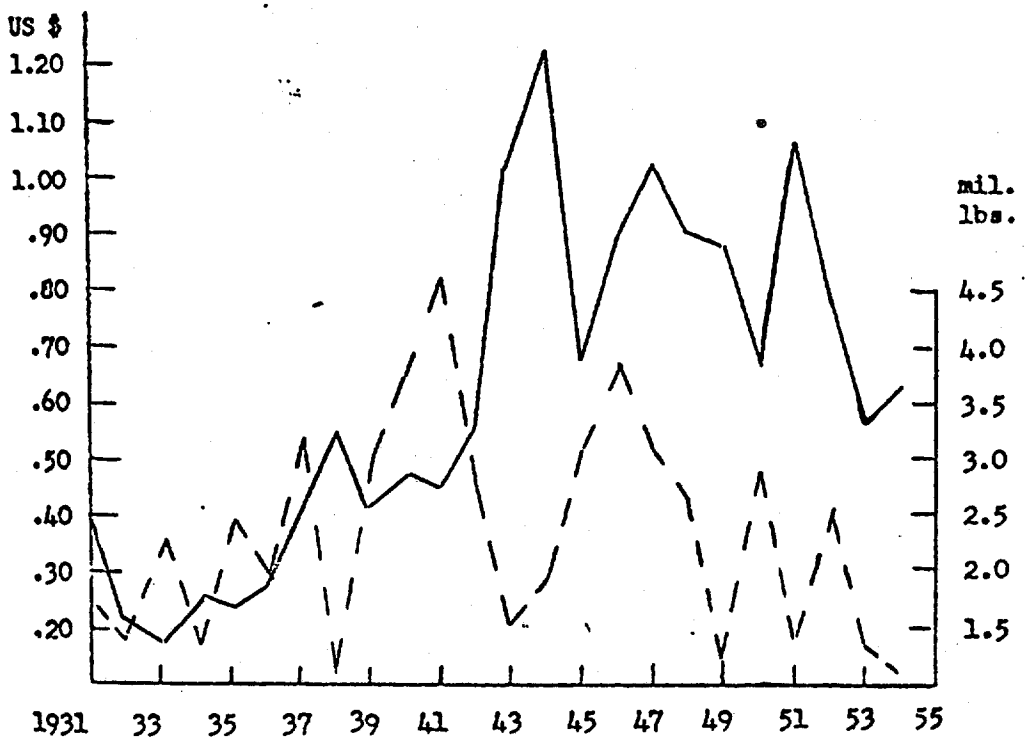

FIG. 10. United States imports of gum tragacanth from 1931 to 1955, showing wholesale price and quantity (broken line). Source, Census Bureau, U. S. Dept. of Commerce.

[1] Spot price for 1955 top grades of ribbon in New York, according to Meer Corporation, range from $2.10 to $3.50 per lb., confirming the Isfahan quotations for the same year.

ince of Charmahal supposedly marketed 2,000,000 rials worth of gum in 1954. Other villages apparently rely heavily upon this natural resource for cash. It is a valuable article in the commerce of Iran.

Collecting the gum is a seasonal vocation for nomads and peasants. Many of them gather it from tribal or village areas and sell directly to local merchants. Collection over large areas is handled by contractors who arrange rights of collection with landowners. The contractor recruits workers who tap the plants and receive pay on a weight and gum-grade basis. The contractor sells to the principal merchants of the cities. From range and village the gum is transported by hand and burros to a trucking point, whence it moves to the grading and packing cities, Shiraz, Isfahan and Tehran.

A gum tapper needs a good pair of legs and a strong back. His tools are a light maddox-like blade on a long handle and a small knife with a curved blade. When collecting he carries a bowl or a bag for the pickings. The earth must first be dug away from the tap root under the low rounded crown of thorns to a depth of four to eight inches (Figs. 6 & 7). This entails hard labor as the ground is rather hard and frequently stony. One or two cuts are then made into the upper part of the tap root, deep enough to open the gum cylinder one to two inches long. The plants are widely scattered, and not more than a few dozen are commonly found on an acre. Any given locality usually has two or three species producing different types of gum. This calls for considerable walking about between plants, both to make the initial root exposure and cut and subsequently for repeated returns to collect the exudate.

According to the collectors, the best grades of gum are those of the first three collections or those made within ten days after cutting. After the initial ten or twelve days from cutting, longer periods may ensue between collections, and the quality then deteriorates. At Bajga a tapper working a high quality species reported that others had the right to collect gum from his plants after he had made his initial first three collections. Anybody may collect it, he said. As new series of cut plants are added to the season's number, there is less and less time to revisit the older cuts, so the exudate may wait as much as a week or two before being collected. Animals are said to like the gum, so there are losses from insects, from lizards, from sheep and goats, as well as from wild gazelles, unless the plants are guarded. During the first ten or twelve days, therefore, the tapper guards his subjects well. Afterwards it apparently pays him to open new accounts by tapping other plants rather than to remain guarding the declining returns from diminishing quality and value. However, in Bavanat Valley, where different species are exploited, three weeks was the normal period between collections, but less valuable gum was being collected under different conditions of communal rights and animal interference. Tapping schedules are therefore determined partly by local customs, by the species available, and by circumstances. One tapper at Bajga was observed returning from a day's collection with about one pound of initial gum with a value of 150–175 rials, or $2.00 to $3.50, representing part time for three days. Others reported that they made up to 250–300 rials or $3.33–4.00 per day.

Trease (7) records the burning of tops at tapping time in the Shiraz area of Fars. This practice was not observed there in 1955 nor called to my attention by informers; government conservation measures may have eliminated it. About Karaj near Tehran, however, the plants were topped at tapping time and the

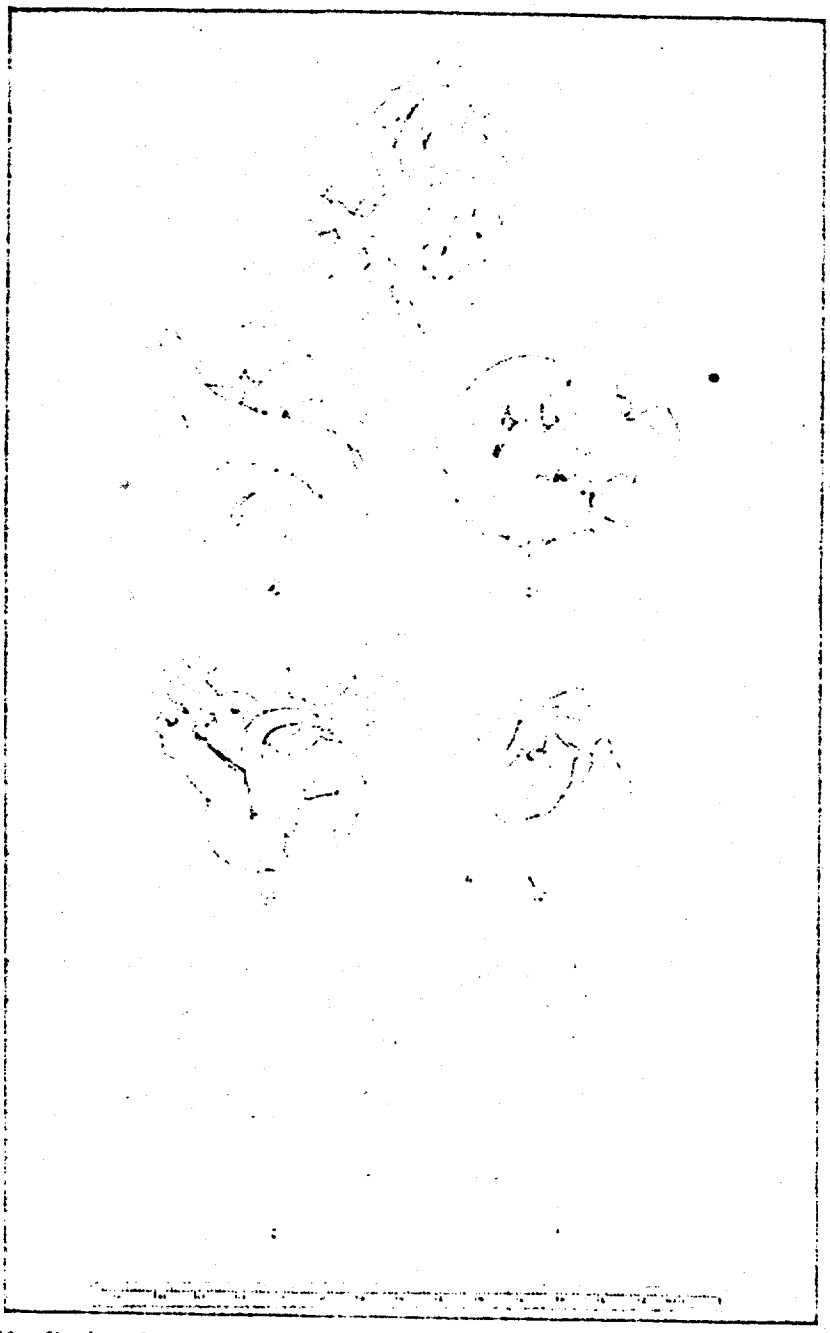

FIG. 11. Grades of gum tragacanth selected according to color and texture; 1, ribbon No. 1; 2, ribbon No. 2; 3, ribbon No. 3; 4, ribbon No. 4; 5, flake grade; and two other commercial gums of Iran; 6, "ghooreh"; 7, "oshagh". (Scale in centimeters).

tappers claimed that they obtained more gum by such operation. It was not observed elsewhere, but these two instances indicate a more general practice. If gum production is actually increased by topping, this would indicate a tie-in with transpiration. However, this appears to be a get-rich-quick makeshift, shortening the harvest season, as topping destroys the manufacturing part of the plant.

Tapping starts in June, and the plants are said to continue to extrude gum until the rains of autumn or for about 100 days or over. If rain falls during the tapping period, it is said to spoil the gum by mixing dirt into it, the plant no longer exudes gum, and the production season is at an end. Therefore, dry summer climates are best suited for gum production. Most of the gum is extruded at night, according to the tappers, and such seems to be true so far as the present authors limited observations are concerned. Presumably exudation pressure increases as transpiration decreases. The plant appears to manufacture gum more or less as it is forced out, the wavy pattern on the exudate reflecting alternation of pressure and its release. It may therefore be regarded as a chemical plant in continuous operation. Table III summarizes the collections of gum samples obtained by the writer in Iran during the early summer of 1955.

Factors Affecting Yield

The quantity and quality of gum yield appear to be affected by many factors. These are outlined below on the basis of field observations. The magnitude of many of them can be determined only by special investigations, and some would require physiological studies under laboratory conditions.

Natural Factors

Kind of plant. The samples collected show that different species and varieties produce various kinds of gum in amounts that differ significantly according to specific identity of the plants. Several species produce top grades of gum, as *A. gossypinus* and *A. microcephalus*, while other species and varieties produce inferior grades.

Exudation deterioration. In general, the gum from any one plant deteriorates in quality as tapping proceeds. The first collections are the best; later collections are assigned to lower grades. This is particularly true of high quality species where collections are made every few days. Species with inferior types of gum, as those tapped on the branches, are generally collected at intervals of weeks, and the gum-quality deterioration is not evident.

Age of plants. The foregoing sample Tables establish that the larger older plants produce greater amounts of gum. Quality according to age appears to be about the same, except in cutting tapped old pockets of gum discolored from previous wounds.

Size of gum cylinder. Generally, the larger the gum cylinder, the better and more the yield. This appeared to be true for quantity throughout all specific and subspecific categories.

Growing conditions previous to harvest. Good spring rains preceding the tapping season were stated several times to lead to good gum production. This would appear to be true on any physiological basis, as soil moisture availability is a vital factor in all plant functions.

Soil moisture at tapping time. A sudden increase in soil moisture during tapping season apparently results in marked decrease of exudation pressure, as rains are said to terminate tapping.

Air humidity at tapping time. Most exudation occurs at night when air humidity is higher than during the day. Humid air would tend to favor less rapid drying of the exudate, with consequent less plugging effect by drying gum and a generally smoother outward flow, or smoother response to pressure.

TABLE III. GUM TRAGACANTH SAMPLES TAPPED AND COLLECTED BY THE WRITER, JUNE–JULY, 1955. AMOUNTS GIVEN IN GRAMS

	Plant size or estimated age	Collected after cutting			Total	Com. grade
		3rd day	6th day	9th day		
A. microcephalus "panbeh" Gentry 15078	med., 3 yrs.	1.75	0.85	0.41	3.01	ribbon
	small, 2 "	1.05	0.21	0.26	1.52	"
	med., 4 "	2.00	0.25	0.21	2.46	"
	large, 8 "	3.70	1.21	0.71	5.62	"
	small, 3 "	1.74	0.55	0.96	3.25	"
	(average)	2.12	0.61	0.51	3.17	
			7th day			
A. microcephalus "ghuchu" Gentry 15164	small		1.24			flake No. 5
	small		2.25			" " 2 & 4
	old bush		1.77			" " 2 & 3
	medium		2.85			" " 2 & 3
	medium		5.06			" " 3
	(average)		2.63			
			6th day			
A. echidnaeformis "panbeh" Gentry 15181	5 plants		unweighted			ribbon
				12th day		
A. clymaiticus "panbeh" Gentry 15048	medium		3.07	2.00	5.07	
	large		3.07			
	medium		2.08			
	large		2.89			
	(average)		3.03			
A. cerasocrenus Gentry 15258	gum cyl. 5–6 mm.	1.08				flake No. 1
	" " 3–4 "	0.50				" " "
		0.27				" " "
		0.14				" " "
	" " 5–6 "	3.14				" " "
	(cuts poorly made account for low yields)					
A. cerasocrenus "gommer" Gentry 15049	large		5.17	3.80	8.97	
	large		2.24	0.38	2.52	
	medium		1.77			
	medium		1.09			
	small		1.46			
	large		1.37 (tapped too high)			
	small		1.32			
	(average)		2.06			
			5th day			
A. cerasocrenus "gommer" Gentry 15080			1.25			
			1.22			
			0.57			
			2.27			
			1.42			
	(average)		1.34			
			7th day			
A. sungunensis "gommer" Gentry 15166	medium		2.62			flake No. 1
	small		0.30			" " 1
	medium		1.40			" " 3
	small		1.13			" " 1
	large		2.31			" " 1 & 3
	(average)		1.55			

TABLE III (*Continued*)

	Plant size or estimated age	Collected after cutting			Total	Com. grade
		3rd day	6th day	9th day		
A. brachycentrus	small		2.16			flake No. 3
"ghineh zard"	small		1.40			" " 2
Gentry 15165	medium		3.94			" " 2
	medium		1.55			" " 1 & 2
	old, gnarled		4.61			" " 1 & 2
	.....		5.30			" " 2 & 3
	(average)		3.16			
			27th day total			
A. brachycentrus	medium					
"ghineh zard"	medium		21.90			flake No. 3, 4, 5
Gentry 15168	(average)		11.00			
			5th day			
A. myriacanthus	branches		2.00			flake
"ghineh cherari"	"		2.96			"
Gentry 15047	"		5.54			"
	"		3.00			"
	"		1.96			"
	(average)		3.12			
			2nd col. 6th day			
A. geminanus "zardeh" Gentry 15182	5 plants		2.84			ribbon No. 5

Air temperature at tapping time. Higher air temperatures during the day increase drying rate of the gum and apparently cause a sealing of the wound during daylight hours when exudation pressure is at minimum due in part perhaps to transpirational loss of moisture. As transpiration ceases, pressure appears to increase until it reaches a point sufficient to unseal the gum-plugged wound, when extrusion resumes.

Animal consumption. Many animals appear to eat the gum, which results in loss of product. The wild sheep of the mountains are said to uncover the roots with their hoofs and to wound the branches with their horns during their rutting season in order to obtain the gum. Aphrodisiac properties are therefore ascribed to the gum. It is more likely, however, that the exudation season of the plant happens to correspond with the rutting season of the sheep. Ants, lizards, domestic sheep and goats appear to be the only serious offenders to the tapper's efforts.

Artificial Factors

Depth and size of cut or cuts. Theoretically, tapping should be done with minimum damage to the plant and to withdraw the gum according to its rate of manufacture by the shrub. The average tapper thinks but little of the former aspect and certainly not at all of the latter. However, tappers informed me in certain cases that the branches should not be tapped or the plant would be killed. In general they make the incision which experience teaches them is necessary to open the central cylinder. Their cuts are usually longitudinal or cross-angled on root or stem, one to two inches long, and three-eighths to five-eighths of an inch deep, the depth of cut depending upon the diameter of root and

gum cylinder. An angled cut is almost certain to cut into the gum cylinder, while a vertical slit may miss it, as occurred in the author's samplings. In time new tissue growth heals the wound around a pocket of reddish old gum. It appears that a hole drilled into the cylinder would be far less damaging. The whole question of cutting technique should be explored with a series of experiments.

Location of cut or cuts on plant. In root-tapped plants the best results are obtained by cuts placed near the top of the root, one to three inches below branching level. Where tapping is done above-ground on stems and branches, the tendency is to make as many cuts as possible, as more gum is thought to be obtained in that way. This obviously causes serious injury, and it is very doubtful that more gum is obtained in that way than by a more prudent cutting pattern carried on over a longer period.

Schedule of gum collection. Removal of the gum or plug should also be done according to the rate of manufacture of gum by the plant. This can be determined only by a series of experiments. For instance, the quality of gum might be better and produced with less injurious effect to the plant by allowing the gum to remain in the wound for many days. However, this exposes the product to dirt and debris carried by wind.

Extent of injury to tops and roots. There is no question that the present method involved in digging earth away from the roots, lopping off the spiny branches that bother the digger or bending them back with his feet, results in serious injury to the plants. The earth is usually dug away from all sides of the root, during which adventitious roots are cut off or injured. Providing for deep-rooted plants by proper culture methods and perhaps preharvest pruning of tops should do much to minimize serious injury during tapping operations.

Relative cleanness of gum. This depends to a large extent upon the habits of the tapper. The tapping excavation should be made deep enough and wide enough to prevent the gum from extruding into the dirt on the sides and bottom of the hole. Adherent particles of earth and rock results in loss of gum and a reduced price. Careful tappers usually place a thorny cover over the excavated basin above the cut, thus protecting the exudate from animals and to a certain extent from rolling earth.

Potential in Cultivation

It appears strange that the source of such a valuable product as tragacanth gum has never been domesticated. Presumably the ancient methods of exploitation have never been seriously questioned. The industry has never graduated from the hunting and gathering stage, which is a fairly primitive anthropological condition. The nomads and the villagers collect the gum according to their customs, while the merchants receive it according to theirs. The pattern has not appreciably changed in over 2,000 years. The Iranian general reaction to the suggestion that the gum plants be cultivated was that there is no need to cultivate, since the plants grow wild. A few entertained the idea as a means of conserving and increasing a natural resource and livelihood. The modern occidentals, who may have considered the plant, were perhaps deterred by the low scale of labor. How could the added cost of cultivation justify itself in the fact of such low returns to labor?

Theoretically, the following considerations appear to favor domestication:

a) An acre of ground would support more than all the plants generally found on a half section of range land. Through cultivation the plants would be brought

into efficient accessibility, eliminating the footwork which consumes most of the tapper's time.

b) Planting in straight rows would make it possible to expose roots with the plow or disc and thus would save the labor of hard digging.

c) Planting on top of borders would lengthen the roots, thereby increasing cylinder volume and yield, and result in much less injury to roots during exposure of them for tapping.

d) Tapping the roots in precise row planting could be done mechanically, or at least with semi-automatic and precise tools.

e) Collection of the gum could also be done on a partly or wholly mechanical basis.

f) Culture and selection of the better varieties would in time mean the maintenance of plants with yields far greater than the present field average of abused and ordinary individuals.

g) The xerophytic and cold-hardy nature of the plants makes them good prospects for relatively non-productive lands in arid and semi-arid climates of the Mediterranean type.

So far as known, the figures in Table III are the only data composed on the amounts of gum produced by tragacanth plants. The following estimate of gum yield is made as a first tentative appraisal.

The weighed samples of *A. microcephalus* at Bajga are the most complete available for any of the species. It is one of the good producers of high quality gum. Moreover, digging and cutting were done by an experienced tapper. After initial release of gum by tapping, gum production by the plant continues at a certain rate. The daily rate can be approximately computed in this case from Table III by adding the average weights of the second and third collections and dividing by the number of days: 1.11 grams by 6, or 0.14 g. per day. Multiplying this daily production rate by the number of days in the tapping season (100) indicates 14.0 g., plus the 2.12 g. of the initial collections, or 16.12 g. as total for the season per plant. The tapper's opinion, incidentally, for the Bajga plants was that they would yield about ten times the amount of the first three lots, or about 30 grams per plant for the season.

A total of 15 grams per plant for the season is here employed in the conservative interests of this estimate. Twenty percent of this is rated as top grades, ribbon Nos. 1 and 2, while 80% is assigned to lower grades of ribbon, the current respective prices of which are about $7.00 and $3.50 per kg. respectively.

Close spacing is accepted as suitable to accommodate the small plants of *A. microcephalus*. If larger spacing should be required, bigger plants with greater yields per plant would result, with but minor readjustment in the calculated yield.

The yield values are for one season only, and apparently should be multiplied by three or four to complete estimate of total return from one planting carried for eight to ten years. The plants are said to be tapped at two years of age and every other year thereafter for six to eight years, the total number

TABLE IV. ESTIMATE OF YIELD AND VALUE FOR ONE ACRE OF *Astragalus microcephalus* GUM FOR ONE SEASON. BASED ON YIELD DATA AND CURRENT MARKET PRICES

Spacing	Plants/acre	Yield, kg. @ 15 g./pl.	20% @ $7.00/kg.	80% @ $3.50/kg.	Yield value
4′ × 1′	10,000	150	$210.00	$420.00	$630.00
3.5′ × 1.5′	8,400	126	175.00	350.00	525.00

tappings on one plant being three or four. This indicates that the plants live for nine to ten years, and would gross $2,000 to $2,400 per acre.

The problems of cultivation and harvest do not appear so formidable as the question of the ability of the plants to grow in cultivation. Mechanics of cultivation are being handled with increasing skill for many distinct kinds of cultivates and their products. But the reactions of the tragacanth wildlings to cultivated soils and microclimates are quite unknown. How will the seed germinate? What diseases will affect them? Are bacteria necessary to their growth, and if so, what are they? Test plantings in various environments would be the first step towards making cultivates of these variable and interesting plants.

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Utilization Abstract

Pine-gum Products. At the U. S. Department of Agriculture's Naval Stores Research Station, Olustee, Florida, "scientists have developed from crude pine gum a chemical, malco-pimaric acid, which has industrial application in printers' inks, paper sizing, alkyd resins, and photographic chemicals. From turpentine, they have prepared many important esters, including several of the esters of pinic acid. These have been shown to be excellent as lubricants for engines of jet aircraft, and as plasticizers—additives that can give plastics such desirable characteristics as low temperature flexibility, durability, and permanence. Another of their turpentine preparations, pinane hydroperoxide, is useful as a catalyst in the production of cold rubber. Addition of metals, such as lead and magnesium, to aldehyde-modified rosin has resulted in improved metal resinates that are valuable as paint driers".

"Now, the Naval Stores Research Station announces the discovery of a new acid in rosin. This acid, named 'palustric' for the longleaf pine—*Pinus palustris*—from which it was first identified, offers promise of greatly extending the usefulness of rosin". Although this acid is a major constituent of pine gum (10%) and of rosin (20%), it was not previously discovered in the century of research on the composition of rosin because it is very difficult to obtain it in a crystalline condition. This acid has already proved useful in the preparation of paper sizing. (Anon., *Chemurgic Digest* 15(10): 4. 1956).

ON THE IDENTIFICATION BY MEANS OF ELECTROPHORESIS AND CELLULOSE ACETATE FOIL DYEING OF GELS & THICKENING SUBSTANCES THAT ARE LEGAL IN SWITZERLAND.

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1. INTRODUCTION

Besides their gelling and thickening properties, gels and thickening substances used in food manufacture or processing also evidence another important characteristic, namely their ability to emulsify and disperse, thereby providing the explanation for their increasingly wide use as stabilizers in the food processing industry.

Article 443 bis of the Federal Food Regulations defines gels and thickening substances as being those capable of yielding aqueous jellies or aqueous, highly viscous solutions already when in low concentrations. (Concerning admissibility of use, see same document, 56, 110, -1956). Even though they are often being used as stabilizers, they are not designated as such in the food regulation. Within the scope of judicial planning concerning food processing in the EEC, namely admissible emulsifiers, stabilizers, gels and thickening substances (WEISS, 1966), stabilizers were defined as such substances suited for maintaining uniform dispersion of two or more immiscible substances. Most gels and thickening substances are included.

Food monitoring efforts have not been wanting to the end of developing practical testing methods for the analysis of gels and thickening substances. Identification is rendered difficult by the fact that these are high-molecular substances for which no easily executed and specific reactions are known, -- except for starch and the alginates. Furthermore they are used in food only in very minute quantities most of the time, because the desired thickening or stabilizing effect often is already achieved for concentrations under 0.5%. The viscometric method (LETZIG, 1934), based on an appreciable viscosity increase of aqueous solutions due to thickening substances, allows an overall indication of any presence of a thickening substance, but does not permit its identification. Various methods are known for qualitative evidence of gels and thickening substances, which among other methods are based on microscopic tests (CZAJA, 1962; BEYTHIEN & DIEMAIR, 1963), on flocculation reactions (LETZIG, 1955) or on paper chromatographic tests of their hydrolysis products (BECKER, 1956; SULSER, 1957; STOLL & PRATT, 1962), that is, of the corresponding monosaccharides. If the gels and thickening substances to be tested are in pure form, they may be easily tested most of the time under the microscope or by the flocculation reaction. If however their presence in food must be shown, in which there is an interfering substance such as egg white material, then the paper chromatographic test of the hyrolysis products is more promising than a flocculation

reaction. However there are cases in which a few gels or thickening substances yield similar sugar components after hydrolysis or where such sugars as glucose and galactose (for instance from lactose in milk products) are detected in the paper chromatographic test, so that the chromatogram will not yield an unambiguous answer. Methods for testing the identity of the purity have been disclosed in the literature (ORGANISATION MONDIALE DE LA SANTE, 1964, 1966). Concerning their numerous applications outside food processing, see also GLICKSMAN (1964).

The process of paper electrophoresis has proved itself for gelatin detection in dairy products (see this publication, 56, 110, 1965). However the cellulose acetate foils are preferable to paper as carrier material, since good separation may be achieved in little time and because the foils may be rapidly rinsed in view of little dye absorption (see section 2.4.1). We further made use of electrophoresis for the analysis of gels and thickening substances belonging to the polysaccharide group.

The described testing method further allows -- besides identification of gels and thickening substances -- quantitative evaluation by means of dye comparisons with solutions of known concentrations of the pertinent gels and thickening substances, the comparison dealing with color intensity. Our research has shown this simple and easily executed electrophoresis process followed by dyeing promises to be very useful in the analysis of gels and thickening means.

As regards food products, polysaccharides may be determined after removal of fatty and egg-white components by means of alcohol precipitation, which is followed by polysaccharide concentration and the subsequently described method.

2. ELECTROPHORESIS OF GELS AND THICKENING SUBSTANCES

2.1 The cellulose acetate foil as carrier substance

Filter paper is unsuitable for electrophoresis research in gels and thickening substances. When the gels and thickening substances being tested are made visible after electrophoresis-separation (see section 3.3), the filter paper reacts as a polysaccharide when the particular dyeing method is used, and it reacts positively. We tested glass fiber strips and cellulose acetate foils in lieu of filter paper. The latter proved particularly suitable. When testing gelatine too, the paper electrophoresis method proved very useful, and cellulose acetate foils were preferable to filter paper as carrier material, because the lesser dye adsorption allowed rapid rinsing of the foils (see figures 1a and 1b).

An important advantage of cellulose acetate foil electrophoresis consists in the shorter test duration with respect to paper electrophoresis, or about 15 to 30 minutes for micro-electrophoresis (section 2.4.2), so that interferences or secondary effects during separation, such as evaporation of buffer liquid and the resulting undesired concentration increase are only of insignificant magnitude.

2.2 BUFFER SOLUTIONS

The conventional buffer solutions for paper electrophoresis may also be used as conducting electrolytic solutions for cellulose acetate foil electrophoresis. In general, less concentrated solutions are preferred for the latter process. We selected a borate puffer with a pH of 10 for the electrophoresis of the gels and thickening substances of the polysaccharide group, however the sodium-carbonate sodium-hydrogen-carbonate buffer is more suitable when testing gelatine (see section 2.4.3). The buffer solution concentrations are so adjusted that for a terminal potential of 200 volts, a current of less than 1 ma/cm of foil width is generated. Using both buffers demonstrated anodic migration in all gels and thickening substances that were tested, so that they must be coated on the cathode side at the beginning of the test.

2.3 RENDERING GELS AND THICKENING SUBSTANCES VISIBLE.

Following electrophoresis migration, the cellulose acetate foil is removed from the electrophoresis chamber and the position of the gels and thickening substances will be rendered visible by dyeing them. The various gels and thickening substances evidencing different chemical properties, four different dyeing methods are required (see section 3), which may be differentiated through dyeing because the gels and thickening substances migrate at the same rate under electrophoresis.

2.4 RESULTS

2.4.1 COMPARISON BETWEEN CELLULOSE ACETATE FOILS AND PAPER STRIPS

Pherograms I and II in fig. 1a show the results of our electrophoresis test with cellulose acetate foils (4 x 30 cm) in the Elphor-H electrophoresis chamber of GRASSMANN & HANNIG. Except for buffer concentration and the kind of depositing of the substance tested, test conditions are the same as for paper electrophoresis. The amounts of carbonate buffer with pH of 10 and ion size of 0.15 microns are diluted with the same volumes of distilled water. Deposition of substance tested takes place - not with a micropipette - but with a stamp consisting of two parallel platinum laminae absorbing each time 6 microliter of liquid.

It will be noted when comparing figures 1a and 1b that the cellulose acetate foil electrophoresis allows good separation between gelatine and milk eggwhite in much shorter a time than is possible for paper electrophoresis (3 hours in lieu of 14). Furthermore, the foils may be rinsed when dyeing much faster also (20 minutes in lieu of 2 hours.

2.4.2 MICRO-ELECTROPHORESIS OF GELATINE WITH CELLULOSE ACETATE FOILS

The BECKMANN-SPINCO microzone electrophoresis system allows executing simultaneously 8 separations for probe quantities of 0.25 microliter solution of substance , corresponding to 2-10 microgram of the substance tested, on one cellulose acetate foil of 5.5 x 14 cm (see section 4). Deposition of substance tested is performed by means of a microstamp. This allows reducing separation time to 15-30 minutes. Normally the separation time

on a cellulose acetate foil 4 x 30 cm in size and with the Elphor-H electrophoresis chamber of GRASSMANN & HANNIG amounts to about 3 hours (see section 2.4.1)

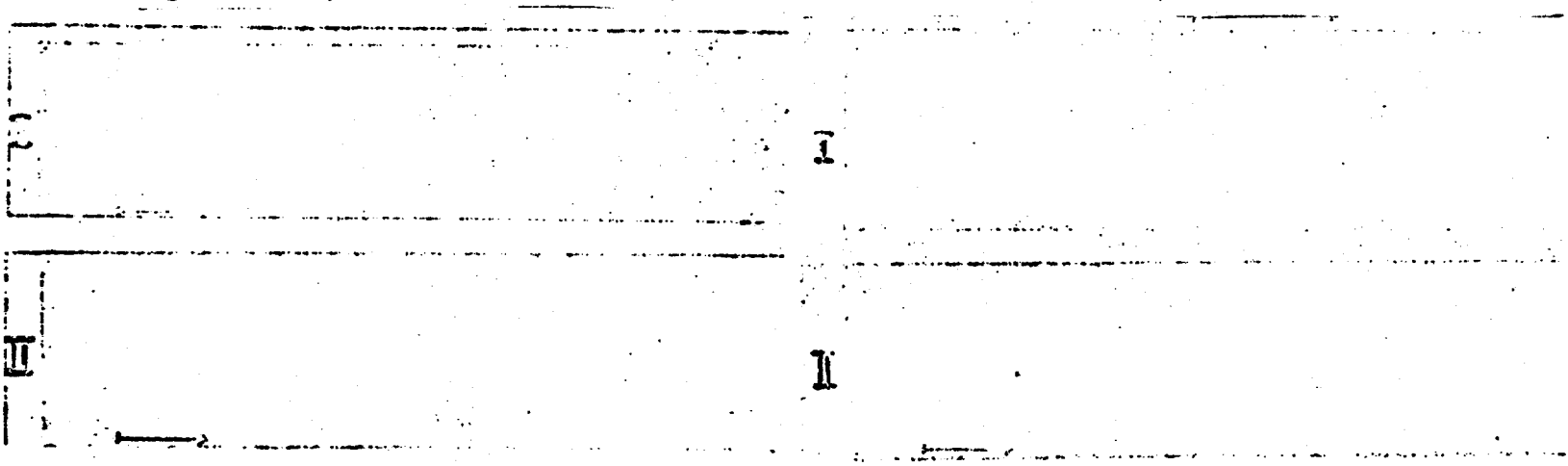


fig. 1a: electrophoresis on cellulose acetate foils

fig. 1b: paper electrophoresis

Fig. 1a: I=gelatin (from 1% gelatin solution, contains about 60 ugm gelatin, deposited with micro-stamp)
II= gelatin and milk egg white (isolated from 0.2% gelatin-content yoghurt, deposited with micro-stamp).
= direction of migration (anodic)
test duration: 3 hours; dyeing plus rinsing: 20 minutes

Fig. 1b: I= gelatin (from 1% gelatin solution, micropipette deposition of 10 ul, contains 100 ugm gelatin)
II= gelatin and milk egg white (isolated from a 0.2%-content-of-gelatin yoghurt, deposited with stamp).
= direction of migration (anodic)
Test duration: 14 hours;
Dyeing and rinsing time: 2 hours

The pherograms show that yoghurt 1 is free of gelatin and that there is gelatin in yoghurt 2. During gelatin isolation, the milk egg white in yoghurt 2 was completely precipitated by means of heat treatment and removed by means of centrifugal action or of filtration. For gelatin concentration, the filtrate will be raised to 10 fold concentration by vacuum evaporation. If the milk egg white is not completely removed, its egg white bands will appear on the pherogram (see picture of self-made yoghurt (b) and that for yoghurt 1). The pherogram shows remaining egg white still capable of migration in yoghurt 1. For the self-made yoghurt sample, -- yoghurt + gelatin --, one may observe completely denatured milk egg white (remaining at the place of deposition) and capable of migrating.

The pherograms therefore show that gelatine tests in dairy products do not require absolute purity in the separated gelatin Even if the extract obtained after yoghurt heat treatment and after gelatin concentration does contain some milk egg white, clean separation of egg whites from gelatin by means of electrophoresis is quite possible.

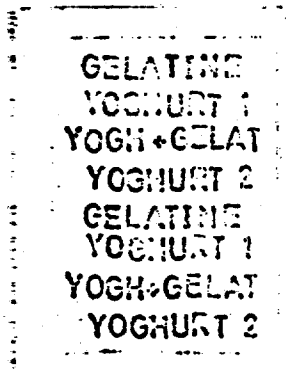


fig. 2

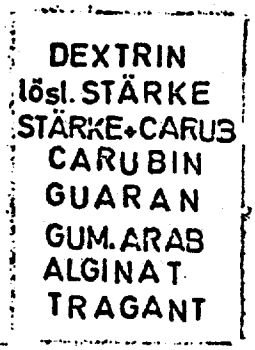


fig. 3
pherogram for PAS positive gels and thickening means.

Fig. 2: shows 8 pherograms from double samples deposited by means of micro-stamp

a) pure gelatin solution as comparison sample; 0.25 ul of 1% solution, corresponding to 2-3 ug of gelatin, deposited
b) extract from self-made yoghurt containing 0.2% gelatin, 10-fold concentration, 0.25 ul deposited, corresonding to 4-6 ug gelatin
c) extracts from commercially obtained yoghurt samples (yoghurt 1 and yoghurt 2); same concentration as in b).

TEST CONDITIONS: 200 volts; carbonate buffer of pH 10 and 0.075 micron ion size; anodic migration; separation time of 25 minutes; amido black dyeing (10 B).

Fig. 3: the amounts deposited are 4-5 ugm for dextrin; 2-3 ugm for soluble starch; 7-8 ugm for carubin; 7-8 ugm for guaran; 7-8 ugm for gum arabic; 2-3 ugm for alginate and 2-3 ugm for tragacanth.

TEST CONDITIONS: 200 volts; borate buffer of Ph 10 and ion size of 0.065 microns; anodic migration; separation time of 15 minutes; PAS dyeing.

2.4.3 MICRO-ELECTROPHORESIS OF GELS AND THICKENING MEANS OF THE POLYSACCHARIDE GROUP

We selected borate buffers with a pH of 10 and ion size of 0.065 microns as the conducting electrolytic solution for the electrophoresis of gels and thickening means of the polysaccharide group. Aside from dyeing, the other test conditions are about the same as those for gelatin (section 2.4.2). Section 4 describes execution of dyeing and of micro-electrophoresis. Fig. 3 shows the pherograms of PAS positive gels and thickening means, fig. 5 shows those made visible following tannin preparation and PAS dyeing, and fig. 6 shows those dyed with toluidine blue O. The pherograms of fig. 4 show the results of electrophoresis separation for mixtures from 2 to 4 different PAS positive gels and thickening means. Section 3 will discuss in detail the dye-affinity of the individual gels and thickening means and their classification according to the dyeing method used.

The pherograms show not only that the gels and thic kening means tested migrate at different rates, but also that they are sensitive in different degrees to dyeing.

For the test conditions as selected, the alginate and the tragacanth spots are subject to a remarkable though constant curvature (see also fig. 5 and 6) the cause of which is unknown to us. For micro-electrophoresis with borate buffer, gelatin too evidences this effect (fig. 5), but this is not the case when carbonate buffers are used (fig. 2).

In the pherograms shown in fig. 3, carubin, guaran and gum arabic evidence nearly the same migration rate. If a longer separation time is used, namely 25 minutes, -fig. 4 -, in lieu of 15 minutes, they may be separated from one another.

If the pherograms of figures 5 and 6 are more closely examined, it will be observed that several gels and thickening means migrate nearly at the same rate. Agar-agar, carubin,and tragacanth in fig. 5, carrageen, sodium pectate and alginate in fig. 6 therefore cannot be separated from one another by means of electrophoresis under the given test conditions. In order to identify them, an improved separation may be achieved as in the case of the PAS positive gels and thickening means (fig. 4) by a longer separation time of 20-30 minutes, and they may be further differentiated because of their variable dye-ability (see table 1,section 3).

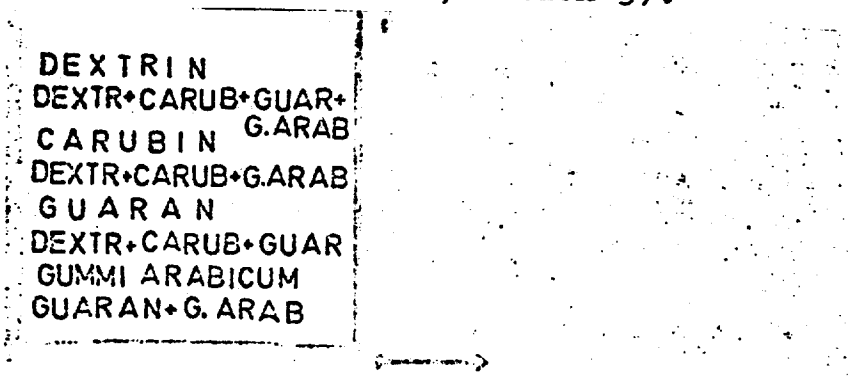


Figure 4

Electrophoresis-separation of mixtures of PAS positive gels and thickening means.

Deposited amount for dextrin was somewhat less (2-3 micrograms in lieu of 4-5), amounts for carubin and guaran were somewhat more (9-10 microgms in lieu of 7-8) than was the case for pherograms in fig. 3. Longer test duration was required for their separation (25 minutes in lieu of 15). Test conditions:

terminal potential: 200 volts
borate buffer: pH = 10; ion size: 0.065 microns
anodic migration
separation time: 25 minutes
PAS dyeing

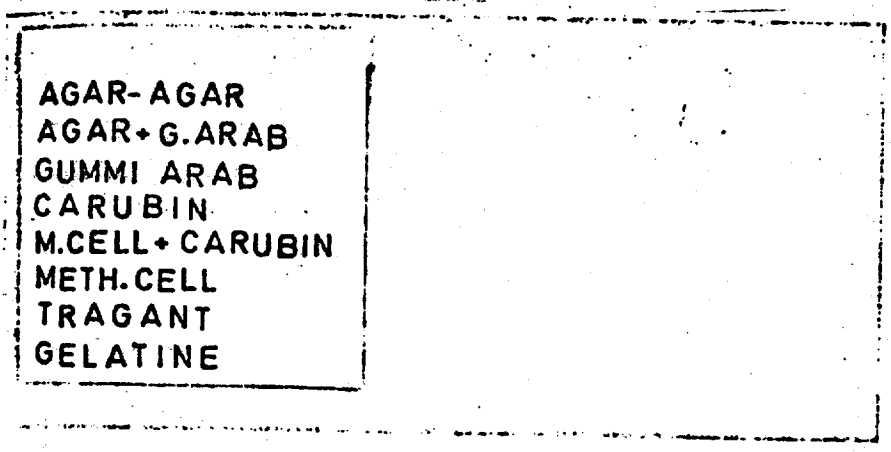
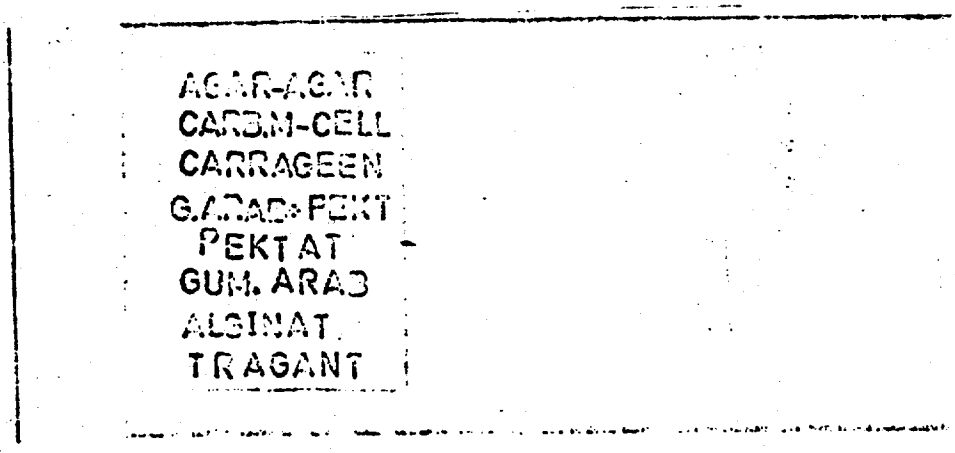


Fig. 5
Pherograms of gels and thickening means rendered visible by means of tannin and PAS dyes preparation.
The deposited amount for agar-agar is 4-5 microgm, for gum arabic 7-8 microgm, for carubin 8-10 microgm, for methyl cellulose 4-5 microgm, for tragacanth 4-5 microgm, and for gelatin 2- microgm.
Test conditions:
terminal potential: 200 volts
borate buffer pH: 10; ion size: 0.065 microns
anodic migration
separation time: 15 minutes
tannin and PAS dyeing preparation*

SUMMARY

The analysis process described enables identification of individual gels and thickening means as such. In the OFFICIAL METHODS OF ANALYSIS of the AOAC (1965) is listed a method allowing separation of gels and thickening means in food products such as ice cream and mayonnaise after removal of fat and egg white components by means of alcohol precipitation. If this precipitation reaction is positive, then electrophoresis and our dyeing method will allow precise identification of the alcohol precipitate.
Quantitative evaluation may take place by comparing with the color intensities of solutions of known concentrations of the particular gels and thickening means. Coarse estimates may be made by glance alone, photometry being appropriate for precise measurements.

Fig. 6
Pherograms of gels and thickening means with affinity for toluidine blue 0



The amounts deposited are, for agar-agar, 4-5 ugm; for carboxy methyl cellulose, 4-5 ugm; for caarageen, 2-3 ugm; for pectate, 4-5 ugm; for gum arabic, 7-8 ugm; for alginate, 2-3 ugm; and for tragacanth, 4-5 ugm.

Test conditions:

terminal potential: 200 volts
borate buffer pH: 10; ion size: 0.065 microns.
anodic migration
separation time: 15 minutes
dyeing: toluidine blue 0

3. DYEING OF GELS AND THICKENING MEANS ON CELLULOSE ACETATE FOILS

3.1 FIXING

Prior to dyeing, the gels and thickening means deposited on the cellulose acetate foils should be first fixed so as not to be dissolved during the dyeing process in the dyeing bath. As other egg white bodies, gelatin may be denatured by drying between 80 and 100°C and therefore be fixed, but this is not recommended for cellulose acetate foils, unfortunately, because of the wear on the foil. We make use of a fixing bath instead of the drying process, such bath consisting of a solution of trichloracetic acid for gelatin and of ethanol for the gels and thickening means of the polysaccharide group.

3.2 GELATIN DYEING

Dyeing of gelatin, which belongs to the protein group, occurs with the conventional egg white substance amido black 10 B, an acid azo-dye. The dyeing process is based on salt formation of the dye's acid groups with the free groups from the egg white. For gels and thickening means from the polysaccharide groups, dyeing must be undertaken in another manner, because there are no free amino groups and therefore do not respond to amido black 10 B (see fig. 7 and table 1).

3.3 PAS DYEING

The PAS (PERIODIC ACID SCHIFF) reaction, recommended by HOTCHKISS (1948) for the dyeing of polysaccharides in animal and plant tissue preparations used in histology, was carried over on our part to gels and thickening means. It was found that not all gels and thickening means could be dyed in this manner. Therefore we make a distinction between PAS positive and PAS negative gels and thickening means.

The Schiff reagency used in PAS dyeing consists of a colorless solution of fuchsin-sulfur acid prepared from adding potassium pyrosulfite and hydrochloric acid to a red, aqueous fuchsin solution. The released sulfur dioxide reduces the red fuchsin to a colorless leuko compound. This Schiff reagency is a known reagency test for aldehyde groups. The gels and thickening means to be dyed first are allowed to react with periodic acid for the generation of aldehyde groups. Two neighboring hydroxyl groups in the polysaccharide molecule will be oxidized to aldehyde groups in the presence of C-C fission. These aldehyde groups react with the Schiff reagency and generate a lilac -red color. Carubin, guaran, soluble starch, dextrin, alginate, tragacanth and gum arabic belong to the PAS positive gels and thickening means. Sodium pectate and carrageen are slightly PAS positive, carrageen's color being very slow to appear.

Polysaccharides, methyl cellulose, cellulose acetates and agar-agar, that lack neighboring hydroxyl groups, correspondingly are PAS negative. Water soluble methyl cellulose shows an average substitution index of 1.64 - 1.92 (GLICKSMAN, 1963) and also contains methyloxyl groups not only as regards the carbon atom C-6 but also to some extent the carbon atoms C-2 and C-3.

For negative PAS and slightly positive PAS gels and thickening means, we found two other ways of dyeing:

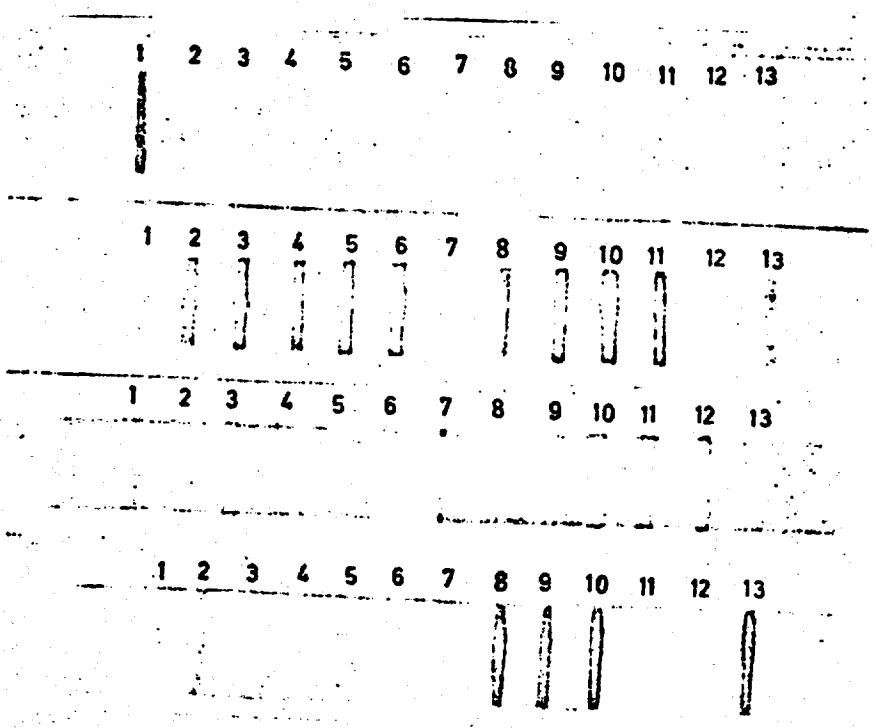
1. a prior treatment with tannin and subsequent PAS dyeing,
2. dyeing with toluidine blue O.

3.4 TANNIN TREATMENT AND PAS DYEING

We first considered using tannin for fixing the gels and thickening means. It was then shown that following such treatment, methyl cellulose could be dyed with the PAS reaction. Previously we had spent much time in vain looking for a way of dyeing methyl cellulose. Besides the latter, gelatin, carubin, agar-agar, tragacanth and gum arabic may also be rendered visible in this fashion. The tannin reaction occurring during this dyeing process -- if so -- is not known to us, except for the corrosive effect.

Besides tannin, we tested many other precipitation means for the polysaccharides, such as lead acetate, phosphor tungsten acid, mercuric chloride and barium chloride, though without satisfactory results obtaining. It was solely when pre-treating with barium chloride that better dyeing of carboxymethylcellulose was achieved. However toluidine blue O provides more sensitive dyeing.

Fig. 7
Dyeing of gels and thic kening means on cellulose acetate foils



Numbers 1 through 13 refer to the gels and thickening means in table 1 below. In this dyeing test, the samples were deposited by means of a stamp and about 1.5 cm apart on a membrane supported from a frame; membrane size was 2.5 x 30 cm. Each amount deposited contains about 6 ul of a 1% solution, that is, about 60 ugm of the particular gel or thickening means. Execution of dyeing is described in section 4.4.

3.5 DYEING WITH TOLUIDINE BLUE O

Toluidine blue O is a thiazine dye used in histochemistry for dyeing acid mucopolysaccharides such as heparin and chondroitin sulphates (RIENITS, 1953). Polysaccharide affinity for toluidine Blue O assumes the presence of acid groups in the molecule. Those gels and thickening means that may be dyed with toluidine blue O, that is, sodium pectate, alginate, tragacanth, gum arabic, carboxymethylcellulose and carrageen, contain either uronic acids or sulfate groups in the molecule.

3.6 RESULTS

By making use of the four different dyeing processes, all gels and thickening means admitted under article 443 bis of the Swiss Food Regulations may be dyed differently on cellulose acetate foils (fig. 7 and table 1).

Table 1
Affinity of gels and thickening means on cellulose acetate foils

Gelier- und Verdickungsmittel	Amido-schwarz 10B	PAS-Anfärbung	Tannin-behandlung + PAS-Anfärbung	Toluidin-Blau O.
1. Gelatine	++	–	(+)	–
2. Na-Pektat	–	(+)	–	(+)
3. Carubin* ××	–		–	–
4. Guaran* ××	–		–	–
5. Lösliche Stärke	–		–	–
6. Dextrin	–		–	–
7. Agar-Agar	–	–		–
8. Carrageen	–		(+)	–
9. Alginat	–	–	–	–
10. Tragant	–			–
11. Gummi Arabicum	–			–
12. Methyl-Cellulose	–	–		–
13. Carboxy-Methyl-Cellulose	–	(+)		–

++ high affinity
+ good affinity
(+) slight affinity
– no affinity

From the dyeing results for the gels and thickening means tested and listed in fig. 7, a listing is made in table 1 where the dyeing methods of the four different kinds used are referred to by plus or minus signs for easier visualisation.

4. PROCEDURE

4.1 SPECIAL EQUIPMENT

BECKMAN-SPINCO microzone electrophoresis system***, consisting of:

microelectrophoresis cell
micro-sample depositing stamp (0.25 ul sample)
cellulose acetate foil (also designated as membrane), 5.5 x 14 cm for 8 samples at a time
power supply (potential: 0-500 volts, 0-50 ma)

FOR ELECTROPHORESIS ON LARGE CELLULOSE ACETATE FOILS:

Elphor-H electrophoresis chamber of GRASSMANN & HANNIG, with associated rectifier and regulating transformer (Bender & Hobein, Munich, Zurich)
membranes of 4 x 30 cm (Schleicher & Schuell, AG, Feldmeilen)
sample depositing-stamp, about 6 ul sample (Kontron AG, Zurich)

REAGENTS:

borate buffer (pH=10, ion size = 0.13 microns): 12.37 gm (= 0.2 mol) boric acid in 100 ml 1-n NaOH solution, completed with distilled water to 1 liter. Then mix 600 ml of this solution with 400 ml of 0.1-m NaOH.

sodium carbonate, sodium hydrogen carbonate buffer (pH=10, ion size = 0.15 microns): mix 750 ml 1-m sodium carbonate solution with 750 ml of 0.1-m sodium hydrogen carbonate solution and with 500 ml distilled water.

PERIODIC ACID SOLUTION: 2 gm periodic acid in 10 ml distilled water solution, then mix with 90 ml 96% vol. ethanol.

TRICHLORACETIC ACID: 5% aqueous solution

TANNIN SOLUTION: 10% aqueous solution ****

SATURATED AMIDO BLACK 10 B SOLUTION: about 0.1 gm amido black in a mixture of 9 parts volume methanol and 1 part volume glacial acetic acid, dissolution through repeated shaking. Solution must be filtered prior to use.

SCHIFF's REAGENT: 1 gm fuchsin in 100 ml distilled water, hot dissolution, cooling to about 50°C; mixing with 1 ml concentrated hydrochloric acid and 2 gm potassium pyrosulfite, hard shaking, rest over night. Shaking with about 1gm charcoal prior to use and filtering.

TOLUIDIN O SOLUTION: 0.2% aqueous solution

formaldehyde-AMMONIA MIXTURE: mix 1-m formaldehide solution with 1-m amoonia hydroxide solution in equal parts volume.

ALCOHOL-HYDROCHLORIC ACID MIXTURE: mix 1 part volume 1-n hydrochloric acid with 2 parts volume denatured alcohol (aceton spirit).

METHANOL - GLACIAL ACETIC ACID MIXTURE: 1 part volume glacial acetic acid with 9 parts volume methanol

TRANSPARENCY SOLUTION: 1 part volume glacial acetic acid and 3 parts volume methanol (always fresh).

methanol-SOLUTIONS OF TESTED GELS AND THICKENING MEANS: 1% solution of individual gels and thickening means in the borate buffer. Exception: a concentration of only 0.3% for agar-agar, because a 1% solution may already cause gel binding. As regards carubin, guaran and tragacanth, only about 20% of the substance in borate buffer goes into solution.

4.3 EXECUTION OF MICRO-ELECTROPHORESIS

4.3.1 FILLING THE MICRO-ELECTROPHORESIS CELL WITH BUFFER SOLUTION

The micro-electrophoresis cell consists of the buffer containers, of the electrode chambers, of a support frame for tensioning and a membrane, of a cell-cover and a cell upper part with slits and grooves for the micro-depositing stamp when depositing samples. Upon removing the cell cover, the cell upper part and the support frame, the siphon located between the electrode chambers will be made horizontal by means of one finger and the cell will be filled through the siphon opening to a height between the lines FLUID LEVEL with buffer solution. When testing gels and thickening means of the polysaccharide group, borate buffer with a pH of 10 and ion size of 0.065 microns is used, and for the gelatin test, a buffer of sodium carbonate / sodium hydrogen carbonate with a pH of 10 and ion size of 0.075 microns. The buffer solutions described in section 4.2 to that end will be first diluted with distilled water in equal parts volume. Following filling of the micro-electrophoresis cell, buffer drops on the cell wall above the buffer level are carefully removed by means of filter paper in order to avoid secondary contacts of electrical current beyond the membrane.

4.3.2 EMPLACING THE MEMBRANE

The membrane first is slightly wetted with the non-diluted buffer being used (see section 4.2). In order to wet evenly, the membrane is placed flat on the surface of the buffer solution. After wetting, the foil is dipped into the buffer solution by means of tweezers. White air inclusion spots are thus avoided. The wet membrane then is removed and easily compressed between two sheets of thick filter paper.

Next the evenly wetted membrane is so tensioned in the supporting frame that all the pegs of the latter fit into the membrane's holes, and therefore the membrane will be held equally tautly everywhere. The supporting frame with tensioned membrane will be so inserted in the micro-electrophoresis cell that the reference hole of the foil will precisely coincide with the numeral 1 of the numbers marked on the cell upper part. This aids remembering the sequence of the deposited samples.

4.3.3 SAMPLE DEPOSITION

After emplacing the cell upper part, deposition of samples may begin. By means of a glass rod, a drop of the solution to be tested is put on a glass plate that is kept neat. The micro-sample depositing stamp by means of its platinum laminae touches the sample drop and the liquid film so created is deposited on the membrane, the platinum laminae remaining about two seconds in touch with the membrane. Prior to the next sample deposition, the platinum laminae of the micro stamp will be rinsed with distilled water and dried by means of careful dabbing with filter paper. Once all eight samples have been deposited, the cell cover is put into position and electrophoresis migration may begin.

4.3.4 ELECTROPHORESIS MIGRATION

The micro-electrophoresis cell is so connected to the power supply that the location of sample deposition is on the side of the cathode. Sample migration is toward the anode. Terminal potential is set to 200 volts. For the buffer concentration used, the current density does not exceed 1 ma/cm of foil width (= 5.5 ma), which is a good value for the migration and separation of the samples that were tested. Upon termination of the desired test duration, which may range from 15 to 30 minutes, the current is shut off, the electrophoresis cell is opened, the membrane is removed and then dyed. Dyeing takes place as described in section 4.4.

4.4 DYEING OF GELS AND THICKENING MEANS

The four different dyeing methods will be discussed in the sections below.

4.4.1 AMIDO BLACK 10 B DYEING

This method is used for rendering visible gelatin besides other egg white bodies. In order to fix the egg whites, the membranes will be immersed for 2-3 minutes in 5% trichloracetic acid solution. Then dyeing takes place for 10 minutes in the saturated amido black 10 B solution. Bleaching of that part of the membrane not carrying egg white takes place when the foil is immersed in a mixture of methanol and glacial acetic acid. The solution will be decanted after 2-3 minutes and replaced by a fresh one. Following threefold washing, white foils are obtained, on which gelatin spots and possibly others appear dyed deep-blue. The foil is rendered transparent in accordance with section 4.5.

4.4.2 PAS DYEING

This method is used to render visible carubin, guaran, soluble starch, dextrin, alginate, tragacanth and gum arabic. The membrane is inserted for 5 minutes in a periodic acid solution. This results in oxidation and also simultaneously in fixing by means of the alcohol of the periodic acid solution. Thereafter the membrane is immersed for 10 minutes in Schiff's reagent so that the individual spots of the tested gels and thickening means appear dyed lilac-reddish. In order to remove the excess Schiff reagent, the membrane is placed for one minute in a mixture of formaldehyde and ammonium, so that solution and membrane become intensely red because of the released fuchsin. In order to bleach that part of the foil free of gels and thickening means, the foil will be rinsed first with denatured alcohol and then several times with the mixture of alcohol and hydrochloric acid. The colortone of gels' and thickening means' spots changes from lilac-red to red-violet. The foil is rendered transparent in accordance with section 4.5

4.4.3 PRE-TREATMENT WITH TANNIN AND PAS-DYEING

This method is mostly used for rendering visible methyl celllulose and agar-agar. Other gels and thickening means that may also be dyed in this manner are gelatin, carubin, tragacanth and gum arabic. The membrane is placed for 7-8 minutes in the 10% tannin solution and then for 5 minutes in the periodic acid solution. Upon periodic acid treatment, foil and solution turn brown. The brown solution is decanted and replaced by new periodic acid. Dyeing proceeds as described in section 4.4.2

4.4.4 TOLUIDINE BLUE O DYEING

This method is mostly used for rendering visible carrageen, carboxymethylcellulose and sodium pectate. Other gels and thickening means that may be dyed with toluidine blue O are alginate, tragacanth and gum arabic. The membrane is placed for 10 minutes in the dye solution. Then the major part of the excess dye is removed by compressing the dyed foil between two sheets of filter paper. Following rinsing with ordinary tap water, the membrane is air dried.

4.5 RENDERING FOILS TRANSPARENT FOR PHOTOMETRIC EVALUATION

Following dyeing, the membrane is immersed for 2-3 minutes in methanol. Then it is placed on a glass plate or disc and subjected for 30 seconds to a fresh mixture of 3 parts volume methanol and 1 part volume glacial acetic acid. Glass plate and membrane are removed from the methanol / glacial acetic acid bath. Upon removing the excess liquid with a rubber sponge or filter paper, they are dried at 110°C for five minutes in an oven. The now transparent pherograms may be kept on the glass plates. They may be carefully removed from the glass plates for the purpose of photometric evaluation and they may be placed in glassines or the likes. The foils treated with toluidine blue O are not subjected to the methanol / glacial acetic acid bath because the spots of the tested gels and thickening means would dissolve, except for carrageen.

Gratitude is expressed to Herr Wenger, chemist from Bern, for verification of this work.

* Only a part hardly visible in this reproduction of the deposited agar-agar migrates on the cellulose acetate foil because of electrophoresis. The component initially remaining is more visible. If dissolved in 0.1-n hydrochloric acid and subsequently neutralized, agar-agar lends itself to easier deposition and will be rendered more easily visible than when in a buffer solution. It was also further shown that for pre-treating agar-agar with tannin, a 0.1% alcohol tannin solution is better suited than the reviously used 10% aqueous tannin solution.

** Carubin and guaran dissolve only to 16-19% in borate buffer. The insoluble part was subjected to centrifugation. Depending on quality, dye affinity of carubin will be more or less pronounced. It is strongest for carboxymethyl-carubin; it is very slight for carubin-fleur. Affinity as a function of quality and origin is being tested for further commercial products.

*** Representative: Kontron AG, Zurich

**** A 0.1% alcohol solution gave better results in more recent tests.

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HYPOCHOLESTEROLEMIC ACTIVITY OF MUCILAGINOUS POLYSACCHARIDES IN WHITE LEGHORN COCKERELS. B.A. Riccardi* and M.J. Fahrenbach*, Biochem. Res. Dept., Lederle Labs., Pearl River, N. Y., and W. C. Grant, Dept. of Physiol., Sch. of Med., Univ. of Miami, Coral Gables, Fla.

Various mucilaginous polysaccharides were found to inhibit the development of hypercholesterolemia in cockerels when incorporated at levels up to 3% into a basal diet (20% casein-8% gelatin-61.3% sucrose) supplemented with 1% cholesterol. Two agents, guar gum and carrageenan, affected approximately a 50% reduction in plasma cholesterol levels. Of less activity were locust bean gum, tragacanth gum, pectin and karaya gum. Mucilaginous substances with low order of activity were salep root, whole psyllium seed husks, shiraz gum and ghatti gum. Polysaccharides such as alginic acid, gum acacia and various dextran preparations were inactive at 3% of the diet. Apparently hypocholesterolemic activity is not solely related to the mucilaginous property of the polysaccharides. Guar gum showed some hypocholesterolemic activity when incorporated at a level of 3% into a natural diet supplemented with 3% cholesterol, although control plasma cholesterol levels were not as high as those obtained with the semi-synthetic diet. No gross toxicity as measured by changes in survival, body weight or total food consumption, was observed with any of the mucilaginous polysaccharides.

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Growth Inhibitory Effect of Certain Polysaccharides for Chickens

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IT IS well-known that the growth of chickens is inhibited if their diet contains either linseed meal (Kratzer and Williams, 1948; MacGregor and McGinnis, 1948), or ground carobs (Kratzer and Williams, 1951; Bornstein *et al.*, 1963), or guar meal (Borcher and Ackerson, 1950; Vohra and Kratzer, 1964). In each case, the feces of the chickens were extremely sticky and tended to paste up the vent. Linseed meal loses its growth-inhibitory properties if it is given a preliminary water treatment; or if the pyridoxine content of the diet is increased (Kratzer and Williams, 1948; MacGregor and McGinnis, 1948). Similar treatment of ground carobs

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either very slight but significant (Lister and Williams, 1951), or no growth improvement (Bornstein *et al.*, 1951) of chicks. Our unpublished studies indicate that guar meal is not improved either by treatment with water or by the addition of pyridoxine.

Linseed meal, carob and guar seeds are excellent sources of polysaccharides which are classified as gums and mucilages. An obvious question in considering these materials is whether this growth depressing effect is due to the high levels of polysaccharides which they contain.

A part of the growth inhibitory properties of linseed meal has been attributed to its mucilage content (Mani *et al.*, 1949; Clagett *et al.*, 1955) even though the latter workers doubted this in another report (Schlamb *et al.*, 1955). A depression in the growth of rats has been observed when their diets contained more than 5% agar agar or Irish moss (Nilson and Schaller, 1941). The digestibility of agar agar, pectin and guar gum is reported to be 21%, 12% and 76% respectively, and no significant depression in the growth of rats was observed when the diets contained these polysaccharides at levels of 15%, 10% and 6%, respectively (Booth *et al.*, 1963). Ershoff and Wells (1962) found no significant depression in the growth of rats which were fed 10% pectin, guar gum, locust bean gum or carrageenan. Carrageenin appears to interfere with the digestion of proteins by pepsin under certain conditions (Vaughan *et al.*, 1962). The present work which was reported in abstract form (Kratzer and Vohra, 1963) is a study of the effects of feeding polysaccharides on the growth of chickens.

EXPERIMENTAL

Commercially prepared samples of the following polysaccharides were used in this study: guar gum,[1] locust (or carob) gum,[1] gum arabic,[1] gum ghatti, gum tragacanth,[1] gum karaya, pectin, carrageenin, cellulose,[2] methylcellulose, sodium salt of carboxymethylcellulose, agar agar, dextrin, dextran and polygalacturonic acid.[3]

Dried okra powder was prepared by homogenizing fresh okra in a blender and an excess of methanol was added to the homogenate. The mixture was stirred and filtered on a Büchner funnel under vacuum. The solid residue was dried in a current of air at room temperature, and ground.

To obtain mucilage, linseed oil meal was soaked in an excess of water overnight, stirred and filtered through muslin. The volume was reduced by leaving the mucilage in shallow pans in a draft of air. The polysaccharide was precipitated from the mucilage solution by the addition of methanol. It was filtered and dried in a current of air at room temperature.

Caramel was prepared by the heating of sugar crystals (sucrose) in an iron crucible over a burner.

Psyllium husk and dried kelp were obtained from commercial sources.

The polysaccharides were added to the stock diet (Table 1) at the required level without making any adjustment in its composition (Table 1) at the required level without making any adjustment in its composition. Day-old, Arbor-Acres broiler type chicks were weighed and distributed into groups of approximately equal average weight. They were housed in electrically heated batteries in which water and feed were available *ad libitum*. The chicks were weighed twice every week as groups and individually at the termination of the experiment.

[1] Samples through courtesy of Stein, Hall and Co., Inc.

[2] Through courtesy of Dr. M. A. Joslyn.

[3] Solka-floc, Brown Co., Berlin, New Hampshire.

TABLE 1.—*Composition of the stock diet*

Ingredient	Inclusion, gm./kg.
Ground milo	175
Ground corn	157
Ground barley	200
Bran, wheat	150
Soybean meal, 44% protein	120
Fish meal	75
Meat and bone meal, 50% protein	12
Whey, dried	25
Milk, skimmed, dried, 35% protein	25
Alfalfa meal	40
Limestone, ground	15
Salt*	5
Vitamins†	1

* Contains 0.025 gm. manganese sulfate (70% feed grade).

† Supplies: riboflavin, 1.1 mg.; niacin, 1.1 mg.; Ca-pantothenate, 1.1 mg.; choline chloride, 5.5 mg.; sulfaquinoxaline, 125 mg.; vitamin A, dry (20,000 I.U./gm.), 375 mg.; vitamin D_3, dry (4,500 I.C.U./gm.), 250 mg.; and folic acid, 33.3 μg.; and bran to make 1 gm.

A slurry of 120 gm. guar gum in 1500 ml. of water was reacted overnight at 37°C. with 100 ml. of a solution containing 6 gm. of either Cellulase-100[1], or the isolated enzyme from the sprouted guar beans (Vohra and Kratzer, 1964). The mucilage [illegible] down in this process and a fluid was obtained which was reduced to about half its volume in shallow pans in a current of air at room temperature. This concentrated solution was mixed with 5880 gm. of stock diet and the diet was fed as such without any further drying.

Pectin (240 gm.) was stirred in 2 liters of water to give a thick, viscous mass which was reacted with 4.8 gm. of pectinase in 100 ml. water at 37°C. for about 12 hours. The viscous mass was stirred frequently and yielded a fluid solution which was concentrated to about 800 ml. in shallow pans in a draft of air and mixed with 5760 gm. stock diet to feed the chicks. It was not dried further.

The relative growth value compares the final average weight of the chickens fed the experimental diet with the average weight of chickens fed the stock (control) diet in the same experiment. For the comparison, the relative weight of the group fed the control diet is adjusted to 100.

[1] Through courtesy of Miles Chemical Co.

TABLE 2.—*Relative growth (R.G.) and gm. feed/gm. gain (F/G) of chickens fed various polysaccharides in their diets*

Experiment Series	1	2	3	4	5	
Duration of trial, days	20	21	20	21	20	
Polysaccharide and its level	R. G.	R. G.	R. G.	R. G.	R. G.	F/G
Control	100	100	100	100	100	1.73
Cellulose, 2%	101	92				
Guar gum, 2%	67.4	64		61		
Guar gum, 1%	84.4	80		72.2	79.1	1.79
Guar gum, 0.5%				78.6	92	1.75
Guar gum, 0.25%				89.1	98.4	1.72
Locust gum, 2%	73	73.6				
Locust gum, 1%			96.8			
Locust gum, 0.5%			87.8			
Locust gum, 0.25%			96.5			
Gum tragacanth, 2%	63.8	66				
Gum arabic, 2%	96.5	88.6				
Dextran, 2%	94.8					
Dextrin 2%		103				
Pectin, 4%	88.6	77.5		89.5		
Pectin, 2%	92.4	86.5				
Kelp, 4%			99.5			
Kelp, 2%			99.5			
Actual gain of control, gm.	256	330	332	294	297	

TABLE 3.—*Relative growth (R.G.) and mg. pancreas/100 gm. body weight (P/B.W.), gm. feed/gm. gain (F/G), of chickens fed various polysaccharides in their diets*

Experiment Series	6		7		
Duration of trial, days	20		20		
Polysaccharide, and its level	R. G.	P/B.W.	R. G.	P/B.W.	F/G
Control	100	375	100	342	1.54
Cellulose, 2%	96.8				
Okra, dried, 2%	87.6	470	81.8	462	1.84
Carrageenin, 2%	75	532	75.4	555	1.91
Linseed mucilage, 2%	95.4	370	97.4	—	1.84
Psyllium husk, 2%	87.4	435	81.8	456	1.92
Methylcellulose, 2%	103				
Carboxymethylcellulose, (Na), 2%	96.5	360	95.8	—	1.88
Caramel, 2%	100				
Actual gain, gm.	339		320		
Control			100	474	1.78
Kelp, 2%			114	506	1.59
Agar agar, 2%			110	415	1.72
Gum karaya, 2%			72	432	1.67
Gum ghatti, 2%			104.5	427	1.81
Actual gain, gm.			280		
Control			100	410	1.78
Gum ghatti, 2%			95		1.81
Gum karaya, 2%			70	500	1.92
Agar agar, 2%			102		1.87
Actual gain, gm.			315		

RESULTS AND DISCUSSION

The relative growth of the chickens fed the diet diluted with 2% of cellulose was 92 to 101% of the controls (Table 2 and 3). When the diets contain 2% guar gum, the relative growth of the chickens was 61 to 67.4% of the controls. The depression in growth was probably not due to the dilution of the energy content of the diet by the addition of 2% gum because the addition of 2% cellulose which also diluted the energy of the diet to the same extent, caused little or no growth depression. The addition of the following polysaccharides at a level of 2% to the chicken diets caused a definite depression in growth of chickens: locust gum, gum tragacanth, gum karaya, dried okra, carrageenin, and psyllium husk (Tables 2 and 3). Pectin exhibited a marked depression in chick growth at a level of 4%. At a level of 2%, growth of chickens on diets containing pectin, gum arabic, dextran, dextrin, gum ghatti, linseed mucilage, methylcellulose, carboxymethylcellulose, caramel, agar agar or kelp was of the same order as for control diets or those containing 2% cellulose.

As low as 1% guar gum in chicken diets caused a definite growth depression (Table 2). As the level of guar gum was decreased to 0.5 or 0.25%, the growth inhibition was also proportionately reduced. However, in case of locust gum, a definite growth inhibition of chicks occurred only at a 2% level.

Relation to composition. The variation in growth depressing effect of various polysaccharides prompted consideration of a possible common constituent responsible for this effect. Most of the data on the chemical constitution of these polysaccharides have been taken from the reviews of Smith and Montgomery (1959).

Guar gum and locust gum are neutral

polysaccharides containing 33-36%, and 14-25% D-galactose; and 64-67% and 75-86% D-mannose, respectively. Guar gum was far more growth inhibitory than locust gum at a level of 1% of the diet. D-galacturonic acid content of gum karaya and okra mucilage is 43% and 6-8%. It is also present in gum tragacanth, linseed mucilage, psyllium husk and pectin but the exact amounts are not known. D-glucuronic acid is present to an extent of 16% in gum arabic; and 12% in gum ghatti. D-galactose content of the gums is as follows: arabic, 52%; ghatti, 27%; karaya, 14%; okra mucilage, 79-80%. Linseed mucilage contains L-galactose. D-galactose is also present in gum tragacanth, agar agar and carrageenin. About 8% D-mannose is present in gum ghatti. Gum tragacanth and psyllium contain L-arabinose which is estimated to be 19% in gum arabic, 41% in gum ghatti, 3-14% in okra mucilage and 12% in linseed mucilage. L-rhamnose is present in gum arabic and its content in gum karaya and linseed mucilage is 15% and 29%, respectively. However, no prediction is as yet possible about the growth inhibitory properties of polysaccharides from their chemical constitution.

The growth inhibition is probably not due to the mucilage character of the polysaccharides in general because not all of them have exhibited growth inhibitory properties for chickens in this study. If those polysaccharides which do exhibit growth depression are allowed to be reacted with suitable enzymes, the reaction products cause no depression in the growth of chickens (Table 4). Guar gum (2%) when fed along with an enzyme capable of hydrolyzing it, at a level of 0.1%, overcame the growth inhibition of chickens to some extent. However, the growth of chickens fed guar gum which has been hydrolyzed with the enzyme was of the same order as on control diets. The enzyme was either isolated from the sprouted guar beans or a commercial preparation, Cellulase-100, capable of splitting polysaccharides. Growth inhibition due to 4% pectin was completely overcome if it was first re-

TABLE 4.—*Effect of enzymes on the relative growth (R.G.), mg. pancreas/100 gm. body weight (P/B.W.), and gm. feed/gm. gain (F/G) of chickens fed various polysaccharides*

Experiment Series	8			9		
Treatment and level	R. G.	P/B.W.	F/G	R. G.	P/B.W.	F/G
Control	100	474	1.78	100	410	1.78
Guar gum, 2%	69.4	510	1.58	61.6	620	2.35
Guar gm. 2%+0.1% guar enzyme	79	508	2.17	76.5	474	2.32
Control+0.1% guar enzyme	117	376	1.63	102	—	1.86
Guar gum (2%) reacted with guar enzyme	102.5	386	1.60	95	378	2.0
Control+0.1% Cellulase-100				100		1.91
Guar gum 2%+Cellulase-100, 0.1%				86	356	2.02
Actual gain, gm. (Experimental period)	280 (20 days)			315 (20 days)		
Control	100			100		
Pectin, 4%	61.5			69.2		1.91
Pectin, 4%+0.08% pectinase	84			83		1.97
Pectin, (4%) reacted with pectinase	100			102		1.89
Control+0.08% pectinase	95.5			98.8		1.75
Actual gain, gm. (Experimental period)	294 (21 days)			297 (20 days)		
Control	100					
Pectin, 4%	53.5					
Polygalacturonic acid, 4%	89.8					
Actual gain, gm. (Experimental period)	327 (20 days)					

fed with the enzyme pectinase. When pectin and the enzyme were fed together, the growth inhibition was only partially overcome. The presence of methoxy groups in pectin was essential for its growth inhibition. This is borne out by the fact that polygalacturonic acid when fed at a level of 4% gave a relative growth of about 80% of the controls and the methoxy derivative of this (pectin) had a value of about 54%.

No information, as yet, is available about the exact sequence in which the various sugars are linked in the various polysaccharides. But it appears that as the frequency of branching increases in the molecule, the growth inhibitory properties also increase. Guar gum has D-galactose on every alternate D-mannose molecule in contrast to carob gum which has D-galactose on every 3rd or 4th mannose molecule of the straight chain part of the polysaccharide. Guar gum is more growth inhibitory than locust (carob) gum. Gum arabic has fewer branches and more of the component sugars are arranged as a straight chain. It has little growth inhibitory properties for chicks. In contrast to this, gum tragacanth has many more sugars branching from the straight chain of the polysaccharide and has definite growth inhibitory properties.

The ratio of feed intake/gain for controls and the diet containing 2% guar gum was of the same order (Table 2), but tended to be higher for okra, carrageenin, flaxseed mucilage, psyllium husk and carboxymethylcellulose (Table 3). Not all of these substances were growth inhibitory.

In general, the pancreas weight per 100 gm. body weight (Table 4) tended to be higher for those polysaccharides which had a growth inhibitory property. Chickens fed psyllium husk had an accumulation of fluid in the proventriculus which gave it an abnormal appearance of a sausage similar to ones observed by O'Dell *et al.* (1959). The incidence of this gross abnormality was only in chickens fed growth-inhibitory polysaccharides but was never as serious as for psyllium husk.

SUMMARY

Several naturally occurring polysaccharides depressed the growth of chickens when fed in a nutritionally balanced diet containing soybean meal, cereals, and fish meal. The growth of chickens was inhibited about 25% to 30% by the inclusion of guar gum, locust gum, gum tragacanth, gum karaya, or carrageenin at levels of 2%; or 4% of pectin in their diets. Dried okra or psyllium husk caused a depression in growth of about 15% at a level of 2% inclusion, and cellulose, methylcellulose, carboxymethylcellulose, dextrin, dextran, linseed mucilage, caramel, gum ghatti, agar agar and kelp appeared to be without any deleterious properties. The growth depressing properties of pectin and guar gum were overcome by their treatment with enzymes capable of hydrolyzing them, namely, pectinase and cellulase or a preparation from the sprouted guar beans.

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Isolation of New Fractions From Tragacanth

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INTRODUCTION

Tragacanth is one of the oldest drugs. It was known in the days Theophrastus who described it three centuries before the Christian era. It has been an official drug since 1820.

Tragacanth is the dried gummy exudation obtained by incision from Astragalus gummifer L. and some other species of astragalus. It occurs in flattened flakes, irregularly oblong, more or less curved, marked on the surface by concentric ridges, frequently about 0.5 cm long and 12 cm wide, white or pale yellowish white, somewhat translucent, odourless and almost tasteless. It is sparingly soluble in water but swells into a homogenous adhesive gelatinous mass.

Tragacanth is a national popular non toxic chemical commonly used in confections, known as "Mofatakka" for purposes of adding weight. Lay people take tragacanth in its Jel form when suffering from urticaria and other dermatoses associated with itching and they believe it as "blood purifier."

The common known pharmaceutical preparations are:

(a) Compound tragacanth powder.

(b) Tragacanth mucilage.

CONSTITUENTS:

The chemistry of tragacanth needs further study. According to O'Sullivan (1991) the soluble part-which is the greater portion, is made up chiefly of polyarabinan-trigalactan-geddic acid; this yields on hydrolysis, arabinose, galactose and geddic acid. The insoluble part is mostly bassorin, $(C_{11}H_{20}O_{10})h$; bassorin acted on by barium hydroxide solution forms two isomers, alpha-and beta-tragacanth-xylan-bassoric acid, which on hydrolysis yield tragacanthose, xylose and bassoric acid.

Norman (1931): disagreed with O'Sullivan concerning the nature of the soluble part of tragacanth. Norman separated the soluble constituent by filtering it in dilute solutions and then concentrating it under reduced pressure and precipitating it with alcohol acidulated with hydrochloric acid. Several reprecipitations were made which resulted in a fine white powder very soluble in water. This was called tragacanthin. It was found to be composed of uronic acid and arabinose. This gave rise to the suggestion that a portion of the arabinose is united to uronic acid to form a resistant nucleus, the residue attached by a glycosidic linkage. He found that tragacanthin consists of 94 per cent of a combination of uronic acid and arabinose in about equal amounts.

Rowson (1937) has suggested that bassorin is analogous to pectin in composition, containing complex methoxylated acids. Demethoxylation of bassorin results in the formation of tragacanthin, which lacks the swelling properties of bassorin. He recommended that a bassorin content of not less than 60 per cent, and a methoxyl content of not less than 3.75 per cent, be required for tragacanth. There is present also small amounts of cellulose, starch and protein substances.

The aim of this work is to show whether Tragacanth has truely any antiallergic value, and if it is so, trials may be continued in an attempt to isolate the active principle.

MATERIALS & RESULTS

Crude Tragacanth was finely powdered and put in cachets, each containing 1 gm.

Twenty cases of various allergic dermatoses of both sexes and of various age groups were selected for the purpose of trials of crude tragacanth. At the same time, there was another group of 10 cases of allergic dermatoses, control group, receiving the same cachets but containing starch in the same weight.

The cachets were given as one cachet 3 times daily after meals. The total amount given for each case varied from 30-60 cachets, 30-60 gms of crude tragacanth.

The cachets containing starch were given to the control group in exactly the same manner.

The results of this preliminary trial could be summarised as follows:

(1) Marked improvement occured in 13 cases (Chronic eczema 6, Atopic dermatitis 1, Recurrent urticaria 2, Prurigo 4). Itching started to decrease in most of the cases within the first week of the intake of the preparation. They began to sleep more comfortably at night and the degree of inflammation and infiltration subsided to a good extent within a relatively short period.

(2) Moderate improvement occured in 3 cases (Chronic eczema 2, prurigo 1).

(3) No improvement in 4 cases (Chronic eczema 2, recurrent urticaria 1, atopic dermatitis 1)

The control group of cases did not show any improvement in their conditions all through the period of trial.

These preliminary results encouraged the investigators to proceed further in an attempt to isolate the active principle.

1-ALCOHOLIC EXTRACT

2 Kgms of Tragacanth (Merk) were exhausted with alcohol (96%) on a water bath using 3 litres flask connected to reflex condenser for 48 hours.

The exhaustion and washings were completed till no appreciable colour or precipitate was obtained from the alcoholic extract on the dish.

The alcoholic extract was filtered and evaporated on infra red lamp at temp., 50-60°C and the final trace of alcohol was removed by vacuum over at minus one atmosphere and temp. 50°C.

The percent of the alcoholic extract was 3.3%.

The extract was put in cachets each containing 0.2 gm which is equivalent to 6 gm of the crude material.

2-The residue left was dried on the infra red lamp at a temp. of 50-60°C and the final trace was removed by vacuum over at minus one atmosphere and temp. of 50°C.

The residue was also put in cachets each containing one gram. The alcoholic extract and the residue were tried in 15 cases of various allergic dermatoses as shown in the following pages.

REPORT OF CASES

Case No. (1):

—Y.S. male aged 26.

—Chronic eczema of both lower limbs of 6 months duration with attacks of exacerbation and remission.

—The patient was admitted to the hospital and remained for 8 days under the usual lines of treatment (antiseptics, for the secondary infection, antibiotics, antihistaminics, vitamins; etc.) with no response.

—Cachets of the alcoholic extract were given as one cachet daily (for 17 days).

—Some improvement began to occur one week after the intake of the extract but the condition recurred when the cachets were stopped for 5 days. Improvement occurred again when the cachets were given again 2 cachets daily for further 17 days.

—The end result after a total intake of 51 cachets was moderate improvement.

Case No. (2):

—M. Gb. male aged 52 years.

—Chronic eczema of both hands of 10 years duration with attacks of exacerbation and remission.

—The present attack is of two years duration and it is the severest attack and of more extensive distribution.

—One cachet of the alcoholic extract was given once daily for 15 days. The itching which was severe became much less and this was noticed by the patient 8 days after commencing the treatment. Also the inflammatory process became less marked.

—Exacerbation of the inflammatory reaction and increase of itching recurred When the cachets were stopped for one week. The cachets were then re-administered as 2 cachets daily for further 17 days when itching and the inflammotory reaction almost completely subsided.

—The patient was followed up for further two months with no evidence of recurrence.

—The end result after the intake of a total 49 cachets is considered as good.

Case No. (3):

—M. O. R. male aged 50 years.

— Subacute eczema of both upper and lower limbs of 6 years duration with attacks of exacerbation and remission.

—The patient was admitted to the hospital and remained for 15 days under the ordinary lines of treatment.

—The alcoholic extract was given as one cachet daily for 15 days but the condition got worse. The treatment was continued with two cachets daily for further 15 days with no evidence of improvement.

—The end result after the intake of a total of 45 cachets taken as poor.

Case No. (4):

—M. M. female aged 30 years.

—Chronic eczema of both hands of 12 years duration.

—During the whole period she was never free for more than a month. She tried most of the available lines of treatment with no marked improvement.

—The alcoholic extract was given as 2 cachets daily for 18 days. The improvement was marked and definite the itching almost disappeared although she was allowed to eat all sorts of food to which she was sensitive.

—Then treatment was continued with the residue as 2 cashets daily for 10 days. After which the condition disappeared completely.

—The patient was followed up for 3 months with no evidence of recurrence.

——The end result after a total intake of 36 cachets of the extract and 20 cachets of the residue is considered as very good.

Case No. (5):

—S. M. female aged 22.

—Subacute eczema of both hands of two weeks duration, with a history of 2 previous similar attacks.

—The alcoholic extract was given as 2 cachets daily for 10 days.

—The improvement was marked and the itching disappeared completely although the patient was allowed to eat all sorts of food previously forbidden.

—The result after the total intake of 20 cachets of the alcoholic extract was very good. Further follow up for 10 weeks showed no evidence of recurrence.

Case No. (6):

—T. T. female aged 24.

—Laboratory assistast.

—Chronic eczema of both hands of 6 years duration.

—The condition was present for two years before joining her job as lab. assistant. The condition is persistent for the last 6 months although she received most of the available lines of treatment including cortisone, both systemically and topically and transferred temporarily to another job.

—The alcoholic extract was given as 2 cachets daily for 10 days.

—Improvement started 2 days after the intake of the drug, itching became less and less and the fissuring found markedly decreased.

—The patient was then allowed to eat all sorts of food previously forbidden but this led to slight exacerbation.

—It was then, changed over to residue and given as 2 cachets daily for 10 days.

—The improvement was striking.

—The result after the intake of 20 cachets of the alcoholic extract was good and

Ser. No.	Name	Sex	Age	Diagnosis	Duration	Line of treatment	Tot. No.	Result	Side effect
1	Y.S.	M	26	Ch. Eczema	6 M	A.* Ext.	51	Moderate	
2	M.G.	M	52	Ch. Eczema	10 Y	„ „	49	Good	
3	M.O.R.	M	50	Sub. Eczema	6 Y	„ „	45	Poor	
4	M.M.	F	30	Ch. Eczema	12 Y	A. E.	36	Good	
						R.	20	V. Good	
5	J.M.	F	22	Sub. Eczema	2 W	A. E.	20	Good	tendency to sleep
						R.			
6	T.T.	F	24	Ch. Eczema	6 Y	A. E.	20	Moderate	
						R.	20	V. good	
7	A.A.S.	M	32	Ch. Eczema	3 Y	A. E.	20	Good	tendency to sleep
8	MM.M.	M	60	Sub. Eczema	7 Y	R.†	30	Slight	
9	M.A.R.	M	23	Ch. Eczema	15 Y	R.	20	Nil	
10	A.A.	M	43	Ch.Urticaria	4 Y	R.	20	Slight	
11	M.S.Kh.	M	32	Ch. Eczema	2½ Y	R.	30	Good	tendency to sleep
12	A.H.	F	18	Neuroder-matitis	6 Y	R.	24	V. good	
13	N.H.	M	20	„	3 Y	R.	24	V. good	
14	A.I.	M	37	„	30 Y	R.	24	Moderate	
15	S.S.	F	3	Ch.Urticaria	5 Y	R.	30	Moderate	

*A. Ext.=Alc. Ext. †R. =Residue.

after the intake of the residue found still better.

—Further follow up for two months showed no recurrence. Slight exacerbation occurred when the patient was transferred back again to her original job.

Case No. (7):

—A. A. S. Male aged 32 years (Student).

—Chronic eczema of both hands of 3 years duration.

—The patient used most of the available lines of treatment including corticosteroids both topically and systemically which led only to temporary relief.

—The alcoholic extract was given as 2 cachets daily for 10 days. He noticed definite improvement from the beginning of the 3rd day, the itching got much less and the inflammatory reaction subsided to a great extent.

—The treatment was continued with the 'residue' as 2 cachets daily for 10 days, the improvement was more marked and at the end of the period the lesion almost cleared off. The patient felt sleepy with the last group of cachets.

—The result of the intake of a total of 20 cachets of the alcoholic extract was good and with the residue it was still better.

—Follow up for further 10 weeks showed no evidence of recurrence.

Case No. (8):

—M. M. H. male aged 60 years.

—Nurse in a special clinic of a dentist

—Subacute eczema of both hands of 7 years duration with attacks of exacerbations and remissions. The maximum periods of remission he had for 3 months.

—The residue was given as 2 cachets daily for 15 days, resulting in amelioration of itching from the beginning of 3rd day, the oedema gradually subsided and oozing markedly diminished.

—Improvement continued for 2 weeks after stopping the intake of the cachets. Then he had slight exacerbation. The patient was followed up for further 8 weeks, the improvement was slight with persistence of moderate degree of itching.

—The result is considered as slight improvement.

Case No. (9):

—M. A. R. male aged 24 (Student).

—Chronic eczema of 15 years duration and widely distributed in the upper and lower extremities.

—Itching was severe particularly at night. He tried most of the available lines of treatment including corticosteroids both systemically and locally with temporary relief.

—The residue was given as 2 cachets daily for 10 days with no improvement.

—The end result is taken as failure.

Case No. (10):

— A. A. male aged 43 years.

—Chonic recurrent urticaria of 4 years duration.

—The patient was never free from the urticarial wheals. The rash had no relation to food. There was no gastro-intestinal troubles and the stool analysis showed no parasites or other abnormal findings. He was advised to do further investigations and to be examined by the dentist and E.N.T specialist.

—The residue was given as 2 cachets daily for 10 days.

—At the first visit the improvement was temporary and then the patient stopped to attend for further follow up.

Case No. (11):

—M. S. K. male aged 32 years.

—Labourer.

—Chronic eczema of both hands of 3½ years duration.

—He tried most of the usual antiallergic medicines including corticosteroids both topically and systemically for several times, with temporary improvement.

—The residue was given as 2 cachets daily for 15 days. Marked improvement was noticed from the beginning of the 3rd day. The patient felt sleepy with the medicine.

—Further follow up showed slight exacerbation 3 weeks after stopping the intake of the drug.

Case No. (12):

—A. H. female aged 18 years.

—Neurodermatitis of 6 years duration. She was admitted to hospital and received the usual antiallergic treatment for 2 weeks resulting in temporary relief.

—The 'residue' was then given as 2 cachets daily for 12 days.

—The improvement was slight at the beginning but later on it was marked and the itching disappeared completely, lichenification became much less apparent too. The patient was discharged for further follow up.

Case No. (13):

—N. H. male aged 20 years.

—He is the brother of Case No. (12).

—Neurodermatitis of 3 years duration. He was also admitted to the hospital and was dealt in the same way as the former.

—The 'residue' was given as 2 cachets daily for 12 days. The itching began to get less 4 days after the intake of the cachets and later on the improvement became striking.

—Further follow up showed definite improvement in the symptoms and signs and the patient was discharged to attend later on.

Case No. (14):

—A. I. male aged 37 years.

—Atopic dermatitis of 30 years duration. admitted to the hospital and kept for several days with ordinary measures with slight improvement.

—The 'residue' was given as 2 cachets daily for 12 days after which there was moderate improvement of symptoms.

Case No. (15):

—S. S. female aged 37 years.

—Chronic recurrent urticaria of 5 years duration.

—At first it was found related to eggs, fish, chocolate and banana eating and the condition persisted for the last 3 years irrespective of food.

—Cholecystectomy 3 years ago she had, also appendicectomy one year after that but with no improvement.

—She used most of the ordinary lines of treatment including systemic corticosteroids with only slight and temporary relief.

—The 'residue' was given as 2 cachets daily for 15 days.

—Relief of the urticarial wheals felt from the 3rd day. The condition did not disappear completely but improved to a good degree.

—The patient is still under observation.

TOXICITY TESTS

1. L D(50) of the alcoholic extract was not reached till 90 times the therapeutic dose when injected intra-peritoneally in mice.
2. 150 times the therapeutic dose was given orally for 15 days continuously with no death of mice at all.

SUMMARY

(1) Trials were given to confirm that Tragacanth has an antiallergic therapeutic value. The results were encouraging the further trials were continued.

(2) An 'alcoholic extract' and the 'Residue' left were tried in 15 cases of various allergic dermatoses. Results were moderate with the alcoholic extract but good and in some cases better with the residue.

(3) L D(50) of the alcoholic extract was not reached till 90 times the therapeutic dose when injected intraperitoneally in mice.

(4) 150 times the therapeutic dose was given orally for 15 days continuously with no death of mice at all.

(5) Further trials are going on in an attempt to isolate the active fraction in the Residue.

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